universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Charakterisierung der Bindungskinetik von Anthrachinonen an verschiedene Plasmaprotein-Fraktionen

verfasst von

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides Statt, dass ich, Katharina Sagaischek, die vorliegende Arbeit selbstständig und ohne Benutzung anderer als der in dieser Arbeit angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

Die Arbeit wurde bisher in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegt und auch noch nicht veröffentlicht.

Wien, im Mai 2014

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1. Einleitung

1.1. Antibiotika Resistenz und marine Organismen

In den späten 1960er- und frühen 1970er-Jahren wurde angenommen, dass Infektionskrankheiten aufgrund des höchst erfolgreichen Einsatzes von antimikrobiellen Arzneimitteln nahezu ausgerottet worden waren. Dies stellte sich aber als schwerer Irrtum heraus, da Mikroorganismen 40 danach durch Bildung unterschiedlicher Resistenzmechanismen eine größere Bedrohung denn je darstellen. Heutzutage sind Infektionskrankheiten die dritthäufigste Todesursache in den USA und die zweithäufigste weltweit. Für die meisten dieser Fälle sind unwirksame antimikrobielle Arzneimittel verantwortlich, und die Resistenz mancher Bakterien und Pilze gegen diese Wirkstoffe stellt in unserer Gesellschaft ein ernsthaftes Problem dar. Aus diesem Grund besteht ein großer Bedarf an neuen antimikrobiellen Wirkstoffen. [1]

Die Suche nach neuen, unkonventionellen Vorkommen für bioaktive Naturprodukte hat die Meere als attraktives Feld hervorgebracht. Die enorme Biodiversität von marinen Organismen stellt einen Grund für das Interesse an den Ozeanen dar. [2]

Im Gegensatz zur sehr gut untersuchten terrestrischen Flora und Fauna, galt das marine Habitat im letzten Jahrhundert als chemisch nahezu unerforscht. Da die Erdoberfläche von mehr als 70% Wasser bedeckt ist, liegt in den Ozeanen die höchste Artenvielfalt von Organismen fast aller Stämme vor. Die für die Pharmaindustrie relevanten Naturstoffe aus marinen Organsimen kommen aber nur in sehr geringen Konzentrationen vor, daher wäre für eine Vermarktung ein massiver Abbau von Nöten. Um dem entgegenzuwirken, wäre „Aquafarming“ eine Möglichkeit, bei der gezielt benötigte Organismen gehältert und nach Bedarf verarbeitet werden können. [3]

Die in verschiedenen Studien untersuchten isolierten Naturstoffe aus marinen Organsimen zeigen eine Vielfalt von Wirkungsspektren, von denen sich die antibakteriellen, anti-inflammatorischen, zytotoxischen, fungiziden und immunsuppressiven als potentielle Pharmaka-Eigenschaften besonders hervortun. [4]

1.2. Anthrachinone

Die chemische Gruppe der Anthrachinone beschreibt pflanzliche Anthracen-Derivate mit dem gemeinsamen Strukturmerkmal 1,8-Dihydroxyanthron und zeigt eine Vielfalt von

pharmakologischen Wirkungen wie abführend, antiarthrotisch, antidepressiv und zytotoxisch. [5]

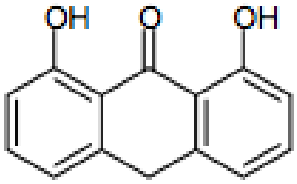


Abb. 1: 1,8-Dihydroxyanthron (www.pharmawiki.at; 13.04.2014)

Altersolanol A zeigt als Naturprodukt aus dem endophytischen Pilz Stemphylium globuliferum, der aus der in Marokko wachsenden Pflanze Mentha pulegium (Lamiaceae) isoliert wurde, cytostatische, cytotoxische, anti-inflammatorische und anti-migrative Aktivität. [6]

Altersolanol A ist ein hydroxyliertes Tetrahydroanthrachinon, das neben anderen isolierten Anthranoiden auch anti-angiogenetische Eigenschaften hat. Es kann auch im endophytischen Pilz Alternaria sp. gefunden werden. Altersolanol A hat an Ring C die meisten Hydroxylgruppen im Vergleich zu den anderen Anthranoiden Macrosporin(1) und einem weiteren Tetrahydroanthrachinon(3) (vgl. Abb.2) und wurde deshalb als einzige Verbindung für weitere Studien selektiert, da die Anzahl der Hydroxylgruppen ausschlaggebend für die Aktivität ist. [7]

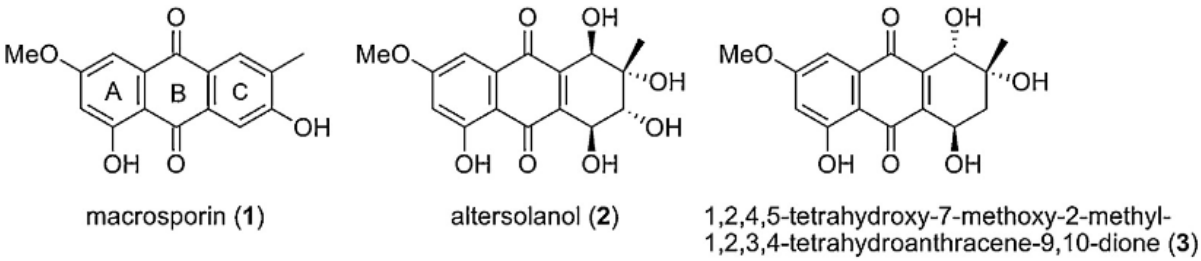


Abb. 2: Isolierte Anthranoide aus Alternaria sp. (Pompeng P., Sommit D., Sriubolmas N., 2013)

Die Firma SeaLife Pharma GmbH aus Tulln in Niederösterreich hat sich als innovatives Biotechnologie-Unternehmen auf marine Organismen spezialisiert und fokussiert die Entdeckung und Entwicklung von neuen bio-aktiven marinen Stoffen. SeaLife Pharma GmbH synthetisiert Anthrachinon-Derivate, deren Vorbilder aus marinen Organsimen stammen. [8]

Die Firma hat neue Anthrachinon-Derivate nach dem Vorbild der Altersolanol-Familie hergestellt, isoliert und charakterisiert. [9]

1.2.1. Doxorubicin

Seit mehreren Jahrzehnten werden unter anderem auch Anthrachinone als wirksame Chemotherapeutika in der Krebstherapie eingesetzt. Bekannte Medikamente aus dieser Gruppe sind die strukturell sehr ähnlichen Wirkstoffe Doxorubicin, Daunorubicin, Idarubicin und Epirubicin, die gegen eine Vielzahl von Tumoren eingesetzt werden. [9]

Doxorubicin ist chemisch gesehen ein Anthracyclin (Anthrachinon-Derivat) und wird therapeutisch als Krebstherapeutikum gegen maligne Tumore eingesetzt. Der Gebrauch wird allerdings durch cardiotoxische Nebenwirkungen limitiert. Der cytotoxische Effekt von Doxorubicin ist durch Interkalation mit der DNA und Topoisomerase-II Hemmung gegeben. [10]

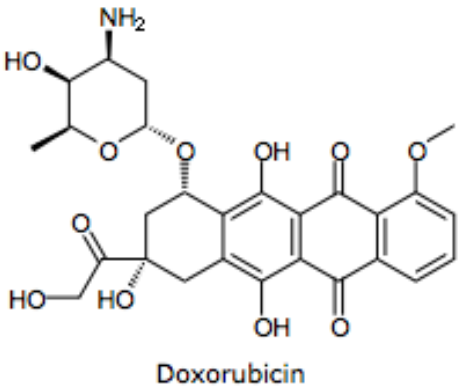


Abb. 3: Strukturformel Doxorubicin (www.pharmawiki.at, 13.04.2014)

Da Doxorubicin ein etabliertes Anthrachinon ist und als Zytostatikum zum therapeutischen Einsatz kommt, sind dessen Daten hinsichtlich pharmakokinetischen Voruntersuchungen bekannt. Daher diente dieser Arzneistoff im Rahmen dieser Arbeit als Modellsubstanz bei der Versuchsdurchführung. Er wurde genauso wie die restlichen Proben behandelt.

1.3. Plasmaproteinbindung

Die Pharmakokinetik als pharmakologische Teildisziplin beschäftigt sich mit den mathematischen Beschreibungen des zeitlichen Verlaufs von Arzneistoffen im Körper unter Verwendung des ADME-Modells. Die pharmakokinetischen Vorgänge, die im ADME–Modell zusammengefasst werden, sind folglich: Absorption, Distribution (Verteilung), Metabolismus und Exkretion. [11]

Wenn ein Arzneistoff vom Körper absorbiert wird, unterliegt er verschiedenen Transport-Prozessen, die ihn zu anderen Körperregionen abseits der Absorptionsstelle befördern. Man spricht von Arzneistoff-Verteilung, der Distribution, die von unterschiedlichen Parametern abhängt und dafür verantwortlich ist, dass sich die Konzentration des vorliegenden Pharmakons ändert. Vornehmlich wird der Arzneistoff durch den Blutfluss in verschiedene Gewebe, Organe und Flüssigkeiten des Körpers verteilt. Die Parameter, welche die Distribution beeinflussen, sind:

- Die Körper-Gewebs Eigenschaften, die die Perfusion betreffen: eine höhere Durchblutung ermöglicht auch eine schnellere Verteilung, sowie eine schnellere Gleichgewichtseinstellung von An- und Abfluten des Arzneistoffes im betreffenden Kompartiment.
- Unterschiedliche Krankheitszustände
- Die Lipophilie des Arzneistoffes: Eine Steigerung der Fettlöslichkeit ermöglicht eine höhere Verteilung ins Fettgewebe.
- Unterschiedliche physiologische pH-Werte
- Das Ausmaß der Protein-Bindung

Um die Konzentration des Arzneistoffs im Körper zu messen, wird meist die Konzentration im Blutplasma herangezogen, wohlwissend, dass diese nicht repräsentativ für andere Gewebsregionen gültig ist.

Ein wichtiger Einfluss-Faktor für die Distribution ist die Bindung des Arzneistoffe an das Gewebe, wie Nuklein-Säuren und ATP, und an Proteine, wie Albumin, Globuline, α-1-azides Glykoprotein und Lipoproteine. Es ist der ungebundene oder freie Anteil, der das Plasma verlässt und wirken kann. Dieser Anteil kann von 0 bis >99% der Arzneistoffkonzentration betragen und ist eine Substanz-spezifische Kenngröße. An Plasmaproteine gebundene Arzneistoffe können nicht pharmakologisch wirken, erst durch Ab-dissoziieren wird das Pharmakon aktiv und kann seine Wirkung entfalten. [12]

Plasma ist der flüssige Anteil des Blutes ohne zelluläre Bestandteile. Die Bindung an Plasma-Proteine ist elektrostatisch, hydrophob, geschieht sehr schnell und ist reversibel. Die verfügbare „binding-site“ dieser Proteine kann eine Sättigung erreichen. Abhängig von Alter und Krankheit kann sich Bindung auch stark ändern. Die Bindung an Plasmaproteine kann die Pharmakokinetik des Arzneistoffes stark beeinflussen. [13]

Alpha-1-azides Glykoprotein und Albumin sind die zwei wichtigsten arzneistoffbindenden Proteine im Plasma. Sowohl saure als auch basische Arzneistoffe binden an Albumin, während alpha-1-azides Glykoprotein nur die Bindung an basische Partner zeigt. [14]

1.3.1. Human Serum Albumin – HSA

Human Serum Albumin (HSA) ist das Protein im menschlichen Plasma mit der höchsten Konzentration, von etwa 40-50g/L. Es wird in der Leber gebildet und ist ein hydrophiles, nicht-glykosyliertes Protein mit einem Molekulargewicht von 55,6 kDa. In seiner Hauptfunktion agiert HSA als Regulator-Protein für den Plasma-kolloidosmotischen Druck sowie als Carrier für viele exo- und endogene Metabolite und Arzneistoffe. Human Serum Albumin macht 60% der gesamten Plasmaproteine aus und seine Blutspiegel sind im Vergleich zu AGP stabiler. [15]

HSA verkörpert den wichtigsten Träger für Fettsäuren und beeinflusst die Pharmakokinetik von vielen Arzneistoffen. Es ist ein wertvoller Biomarker für zahlreiche Krankheiten, wie rheumatoide Arthritis, Ischämie und Krebserkrankungen. [16]

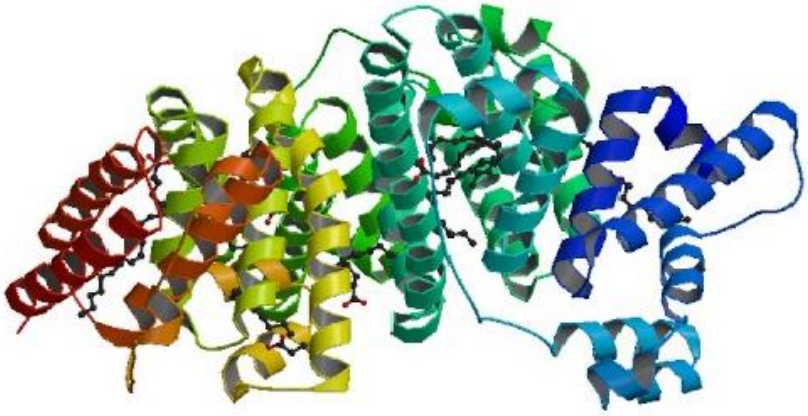


Abb. 4: HSA komplexgebunden mit Arachidonsäure (http://www.pdb.org/pdb/explore/explore.do?structureId=1GNJ)

1.3.2. Alpha 1-azides Glykoprotein – AGP

Das auch im Plasma vorkommende α-1-azide Glykoprotein (AGP) ist synonym zum akute-Phase Protein Orosmucoid (ORM). Es handelt sich dabei um ein Glykoprotein mit einem Molekulargewicht von etwa 40 kDa. AGP wird sowohl in der Leber, als auch in nicht-hepatischen Zellen, wie Granulozyten und Endothelzellen, gebildet. Beim gesunden Menschen kommt AGP in einer Konzentration von ca. 0.77 g/L vor. Seine Konzentration

ist bei Infektionen, Entzündungen und Krebserkrankungen erhöht. Da die AGP Konzentration bei unterschiedlichen Krankheiten erhöht ist, ändert sich dadurch auch dessen Bindungskapazität. [14]

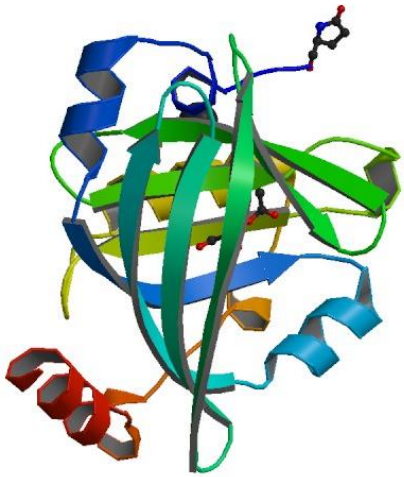


Abb. 5: AGP Struktur
(http://www.pdb.org/pdb/explore/explore.do?structureId=3KQ0)

Auch durch den Gebrauch von Arzneistoffen oder während der Schwangerschaft kann sich die AGP-Konzentration beträchtlich ändern. Das α-1-azide Glykoprotein fungiert als Trägerprotein für basische und neutrale, lipophile endogene Stoffe wie Steroidhormone oder Xenobiotika. [17]

Die Interleukine 1 (Il-1) und 6 (IL-6), sowie Glucocorticoide zählen zu den stärksten Stimuli der AGP Gen-Expression. Die immun-modulatorische Funktion und Wirkung des AGP ist auf den hohen Glykosylierungsgrad zurückzuführen. Über den autokrinen und parakrinen Signalweg ist das alpha 1-azide Glykoprotein in erster Linie in die Regulation der Angiogenese involviert und könnte auch die Gefäßsprossung von Tumoren beeinflussen. [18]

1.4. Untersuchte Substanzen

Im Rahmen der Diplomarbeit wurde die Proteinbindung an die Plasmaproteine HSA und AGP von 14 neuen Anthrachinon-Derivaten und Doxorubicin.HCl untersucht. Die Substanzen wurden, mit der Ausnahme von Doxorubicin.HCl, von der Firma SeaLife Pharma GmbH synthetisiert und bereitgestellt. Als Vorbilder für diese Strukturen diente das bereits in den 1960er Jahren bekannte Altersolanol A, ein Anthrachinon mit

antiinfektiven Eigenschaften. Beim chemischen Grundkörper dieser Verbindungen handelt es sich ebenfalls um ein Anthrachinon.

1.4.1. Chemische Strukturen

Da es sich um eine präklinische Untersuchung handelt, sind die Originalnamen und Strukturen der neuen Anthrachinon-Derivate patentrechtlich geschützt. Weder die Namen, noch die eigentlich Strukturen dürfen in dieser Arbeit veröffentlicht werden. Deswegen dient hier eine allgemeine Grundstruktur des Anthrachinon-Grundkörpers der näheren Erläuterung des chemischen Aussehens. Diverse Reste, in denen sich die einzelnen Anthrachinon-Derivate von SeaLife Pharma GmbH unterscheiden, sind mit –R gekennzeichnet.

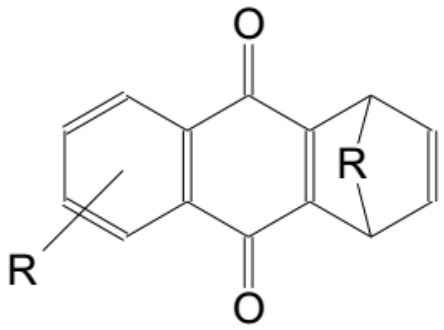


Abb. 6: Grundstruktur der SL-Substanzen (Zeichenprogramm)

Durch den patentrechtlichen Schutz resultierte eine Benennung der 14 Substanzen mit SL1 bis SL14 statt den eigentlichen Namen, wobei SL als Kürzel für die Herstellungsfirma SeaLife Pharma GmbH steht.

Die Arbeit mit den Substanzen geschah nicht chronologisch nach aufsteigender Nummer, sondern nach chemisch zusammengehörigen Eigenschaften, wie z.B. der Löslichkeit und dem Bedarf an unterschiedlichen Eluenten. Deswegen wurden die Substanzen nicht von SL 1 aufwärts bearbeitet, sondern beginnend bei Doxorubicin.HCl als positivem Standard.

Interessanterweise zeigten unsere Substanzen keine Fluoreszenz, obwohl diese für Anthrachinone charakteristisch ist. [19]

2. Rationale

Im Rahmen dieser Diplomarbeit soll für die vorliegenden Substanzen, SL1 bis SL14 und Doxorubicin.HCl als Referenzbeispiel, die Proteinbindung an die Plasmaproteine HSA und AGP getestet werden. Dafür stehen drei unterschiedliche in Vitro Proteinbindung–Kits zur Verfügung. Mit den ersten beiden Kits wird die Bindung der vorliegenden Substanzen sowohl an HSA als auch AGP getestet. Das dritte Proteinbindungs-Kit erlaubt einen Nachweis der Proteinbindung an HSA und AGP gleichzeitig.

Zur Analyse der Proteinbindung wird die HPLC als Methode der Wahl genutzt. Eine Auswertung der Ergebnisse wird mit einer dem Testsystem zugeschnittener Software via Excel durchgeführt.

3. Experimenteller Teil

3.1. Laborausstattung

3.1.1. Chemikalien und Reagenzien

Die für die Laborarbeit benützten Chemikalien und Reagenzien und deren Hersteller sind in der nachfolgenden Tabelle aufgelistet.

Bezeichnung	Hersteller
Acetonitril	Merck KG
Aqua bidest.	Department f. Pharmakognosie, Universität Wien
Dimethylsulfoxid 100% D8418 (100ml)	Sigma-Aldrich
Doxorubicin.HCl, Ch-Nr.: 40157001	Ebewe Pharma (Novartis)
Kaliumhydrogenphosphat 99%	Sigma-Aldrich
PBS Tabletten P4417-50TAB	Sigma-Aldrich
8,5% Phosphorsäure	Sigma-Aldrich
Probensubstanzen: SL 1-14	SeaLife Pharma GmbH, Tulln

Tab. 1: Chemikalien/Reagenzien und Hersteller

3.1.2. Eluenten

Für die Elution der Proben wurde mit drei verschiedenen Eluenten gearbeitet und die Elution war isokratisch.

Bei der isokratischen Elution, im Gegensatz zur Gradientenelution, bleibt die Zusammensetzung der flüssigen mobilen Phase während des gesamten Elutionsvorgangs konstant, damit wird das Gleichgewicht zwischen stationärer und mobiler Phase konstant gehalten. [20]

Damit die Eluenten nach der Herstellung zum Einsatz an der HPLC kommen konnten, mussten diese eine halbe Stunde auf dem Ultraschallbad entgast werden, um ein Entweichen des Luft-Sauerstoffes zu garantieren.

Eluenten-Bezeichnung	Zusammensetzung	Eluierte Substanzen
„Isokratisch 1“	0.8 g KH_2PO_4 in 800 ml Aqua dest. eingestellt mit 8,5 % Phosphorsäure auf pH = 4.4 aufgefüllt mit 1200 ml Acetonitril ≙ dem Verhältnis H_20:ACN von 40:60 v/v	SL1, SL2, SL5, SL6, SL7, SL8, SL9, SL10, SL11, SL12, SL13, SL14
„Isokratisch 2“	0.4 g KH_2PO_4 in 600 ml Aqua dest. eingestellt mit 8,5 % Phosphorsäure auf pH = 4.4	SL 3, SL4

	aufgefüllt mit 400 ml Acetonitril ≙ dem Verhältnis H_20:ACN von 60:40 v/v	
Eluent für Doxorubicin.HCl	0.5g KH_2PO_4 in 500 ml Aqua dest. 250 ml ACN 150 ml MeOH	ausschließlich für Doxorubicin.HCl

Tab. 2: Eluenten und Zusammensetzung

3.1.3. Waschflüssigkeiten

Die Waschflüssigkeiten dienten der HPLC-Anlage, um Schläuche, Zugänge, die Nadel für die Probenentnahme und die Säule zu waschen, damit zwischen Probennahmen und Versuchsreihen ein Mitschleppen und nachfolgende Verunreinigung durch andere Substanzen verhindert werden. Die Waschflüssigkeiten mussten vor dem Anhängen an die HPLC-Anlage eine halbe Stunde lang auf dem Ultraschallbad entgast werden.

- Waschflüssigkeit SL-Substanzen und Doxorubicin.HCl:

  Zusammensetzung:

  H_2O und ACN im Verhältnis von 20 : 80, auf dem Magnetrührer vermischt

3.1.4. Phosphatpuffer

Der Phosphatpuffer dient zur Herstellung der DMSO Lösung.

- Herstellung:

  2 Tabletten der Sorte: Phosphat buffered tablet P4417 Lot# SLBD4322V wurden in 400 ml H_2O dest. gelöst und auf dem Magnetrührer gut vermischt

3.1.5. Lösungsmittel

Die Löslichkeit der Proben war, mit Ausnahme von Doxorubicin, anfangs unbekannt. Als Lösungsmittel zur Herstellung von Stammlösungen der SL-Substanzen und Doxorubicin.HCl diente eine 32% Dimethylsulfoxid (DMSO)-Lösung.

Es wurden 1000ml einer 32% DMSO-Lösung, ausreichend für alle Versuchsdurchführungen, hergestellt. Die Konzentrationsangabe von 32% errechnete sich aus dem User Guide der Test-Kits. (s.3.4.2)

- Zusammensetzung für 1000ml 32%DMSO:

  680 ml PBS Phosphatpuffer

  320 ml 100% DMSO

3.2. Geräte und Materialien

3.2.1. Allgemeine Geräte und Materialen

Bezeichnung		Hersteller
Analysenwaage: Sartorius H110		Sartorius Mechatronics Austria GmbH
Braunglasfläschchen (HPLC)		Biolab
Caps (HPLC): 8-SC		Chromacrol®
Decapper (Transil^{XL} Kits)		Micronic
Eppendorf Pipetten: Eppendorf Research 2-20µl; 100-1000µl		Eppendorf A
Eprouvetten: 100 x 16 mm		Assistent Deutschland
Magnetrührer: MR 3001 K		Heidolph Instruments GmbH & Co. KG
Messkolben: 50ml; 100ml		
Microeinsätze (HPLC Braunglasfläschchen)		Biolab
Pasteurpipetten aus Glas: 150 mm Gesamtlänge		Assistent Deutschland
pH-Meter: ORION 2 STAR pH Benchtop		Thermo Scientific
Pipettensauger		
Pipettenspitzen: Universal blue Tipps, 50-1000µl; Universal yellow Tipps, 2-200µl		VWR®INTERNATIONAL
Schüttler	Plate Shaker: Mix Mate PCB-11	Eppendorf®
	Vortexer	Mixer VELP® SCIENTIFICA
Septum für Sampler-Fläschchen: 8-ST15		Chromacrol® biolab
Transil^{XL} Binding Kits (Mikrotiter-Platten)		Sovicell GmbH
Ultraschallbad: BANDELIN SONOREX RK 150		BANDELIN electronic GmbH Co. KG
Vials (HPLC): p336		Chromacrol®
Waage: Sartorius M-prove AY612		Sartorius Mechatronics Austria GmbH
Zentrifuge: Labofuge 400 FUNCTION Line		Heraeus Instruments GmbH

Tab. 3: Labor-Geräte und Materialien

3.2.2. Analysenverfahren HPLC

Für die Quantifizierung der Proben waren verschiedene Analysenverfahren möglich, unsere Proben sind mit der HPLC analysiert worden.

HPLC steht als Abkürzung für „High Performance Liquid Chromatography“ oder auch „High Pressure Liquid Chromatography“; zu Deutsch „Hoch-Leistungs-Flüssig-Chromatographie“, oder „Hoch-Druck-Flüssig-Chromatographie“ und ist eine äußerst leistungsfähige Trennmethode, deren Chromatogramme unmittelbar qualitative und quantitative Informationen liefern. Die Retentionszeit ist für jeden Stoff eines Gemisches

charakteristisch und die Höhe und Breite eines Peaks proportional zur Menge des entsprechenden Stoffes. Die Substanzen werden in einer Säule aufgetrennt, wobei es sich bei der mobilen Phase um die Probenlösung handelt, und die stationäre Phase an der Säule aufgebracht ist. Bei der HPLC ist ein sehr hoher Druck notwendig, um die Probe durch die Säule zu pumpen, das macht die Methode aber auch leistungsfähiger. [21]

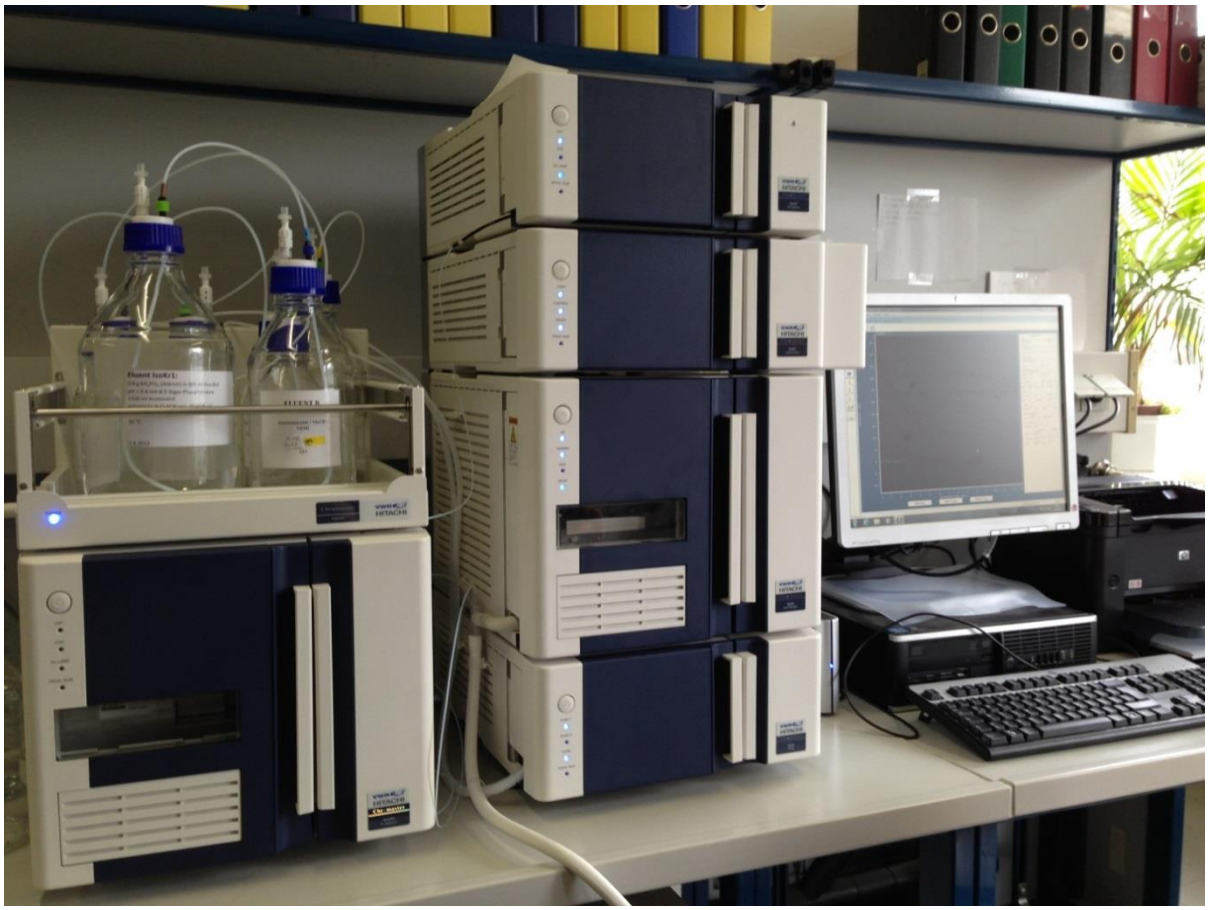


Abb. 7: HPLC Anlage Merck-Hitachi (Foto: Sagaischek K.)

In der folgenden Tabelle sind die Bestandteile der benützen HPLC-Anlage mit deren Bezeichnungen und Einstellungen aufgelistet.

HPLC VWR Hitachi Chromaster	
Pumpe: 5110	Flussrate: 0,8 ml/min
Autosampler: 5210	Temperatur: 10 °C
	Injektionsvolumen: 40 µl
	Druck: 85 bar (Im Schnitt 88 bar)
Thermostatisierbarer Säulenofen: 5310	Temperatur: 36 °C
UV-Detektor: 5410 single wavelength modus	Wellenlänge λ: 270 nm
Organizer	
Kühler: Cooler-Peltier	Temperatur: 16°C

Entgaser	kontinuierliche Entgasung
Software	Chromaster System Manager
PC	HP Compaq 6200 Pro Small Formfactor
Waschflüssigkeit	H_2O:ACN = 20:80 (v/v)

Tab. 4: HPLC VWR Hitachi Chromaster Daten

3.3. Probenvorbereitung

3.3.1. Test-Kit – Vorbereitung

Das für die Untersuchung der Plasmaprotein-Bindung vorgesehene In-Vitro Testsystem Transil^{XL} musste ab dem Zeitpunkt Lieferung bis zur Benützung im Tiefkühlschrank bei -20°C aufbewahrt werden. Vor Gebrauch musste das Test-Kit aufgetaut werden. Es wurde entweder am Vorabend in den Kühlschrank bei 4°C bis zum nächsten Tag, oder 3 Stunden vor Arbeitsbeginn bei 20-25°C Raumtemperatur aufgetaut. Das Test-Kit war betriebsbereit, wenn es Raumtemperatur erreicht hatte.

3.3.2. Stammlösungen

Für jede Proben-Substanz wurde eine Stammlösung mit DMSO als Lösungsmittel bereitet. In den User-Guides, die zum Test-Kit gehören, wurde eine ideale Konzentration von 80 µmol der Stammlösung beschrieben.

Durch das Molekulargewicht der einzelnen Substanzen wurde die für die Stammlösung benötigte Einwaage berechnet.

- Erstellen von 100ml einer 80µM Stammlösung von Doxorubicin.HCl

  Molekulargewicht (MG) = 580 g/mol

  1mol ≙ 580 g

  1µmol ≙ 580 µg |*80

  80µmol ≙ 46,400 µg

  46,400 µg → 46,4 mg/ 1000ml

  → 4,64 mg / 100ml

Allgemeine Formel zur Berechnung der Einwaage: $EW(mg) = MG \times 0{,}008$

Das benötigte Volumen der Stammlösung für die Versuchsdurchführung jeder Proben-Substanz belief sich auf ~400µl. Allerdings war die Einwaage für dieses Volumen zu gering, um genau arbeiten zu können. Aus diesem Grund resultierte ein Stammlösungs-Volumen von 100ml.

Um 100ml einer 80µM Stammlösung von Doxorubicin.HCl zu erhalten, wurden 4,64 mg der Substanz auf der Analysenwaage eingewogen und in 5ml des Lösungsmittels in einer Eprouvette gelöst. Die Löslichkeit der Testsubstanzen variierte sehr stark. Um eine Löslichkeitsverbesserung zu erzielen, wurden die Eprouvetten von weniger gut löslichen Substanzen über eine Apparatur in das Ultraschallbad gestellt.

Anschließend wurde die Lösung auf 100ml 32% DMSO in einem Erlenmeyer-Schliffkolben aufgefüllt. Bei anderen, schlechter löslichen Proben, wurde der 100ml Kolben erneut ins Ultraschallbad gestellt.

Die nachfolgende Tabelle enthält die Informationen zu den Stammlösungen der Proben.

Substanz	**MG (g/mol)**	**Einwaage für 80µM Stammlösung (mg/100ml)**	**Löslichkeit in 32% DMSO**	**Charakterisierung der Probenlösung**	**Dauer am Ultraschallbad**
SL1	240	1,92	schwer löslich	Gelblich	1h
SL2	242	1,94	schwer löslich	Gelb	1h
SL3	395	3,16	leicht löslich	dunkelgelb, hellt etwas auf	30min
SL4	359	2,87	löslich	unbeständig, hell bis dunkel-braun, Schwefelgeruch, Lichtschutz erforderlich	5min
SL5	268	2,14	leicht löslich	Hellorange	30min Eprouv. 10 min Kolben
SL6	282	2,26	leicht löslich	Gelb	-
SL7	404	3,23	löslich	hellgelb, Partikel vorhanden	5min
SL8	376	3,01	leicht löslich	gelb, entfärbt sich nach 3 h	5min
SL9	420	3,36	leicht löslich	Hellgelb	5min
SL10	382	3,06	löslich	hellgelb, entfärbt sich	45min
SL11	395	3,16	leicht löslich	Hellgelb	5min
SL12	270	2,16	schwer löslich	2h Ultraschallbad, hellorange	2h
SL13	703	5,62	kaum/ nicht löslich	60% DMSO: besser löslich, gelb, trüb, hellt auf	4h
SL14	418	3,34	leicht löslich	Hellgelb	5min
Doxorubicin.HCl.	580	4,64	leicht löslich	rot-orange	-

Tab. 5: Eigenschaften der untersuchten Substanzen

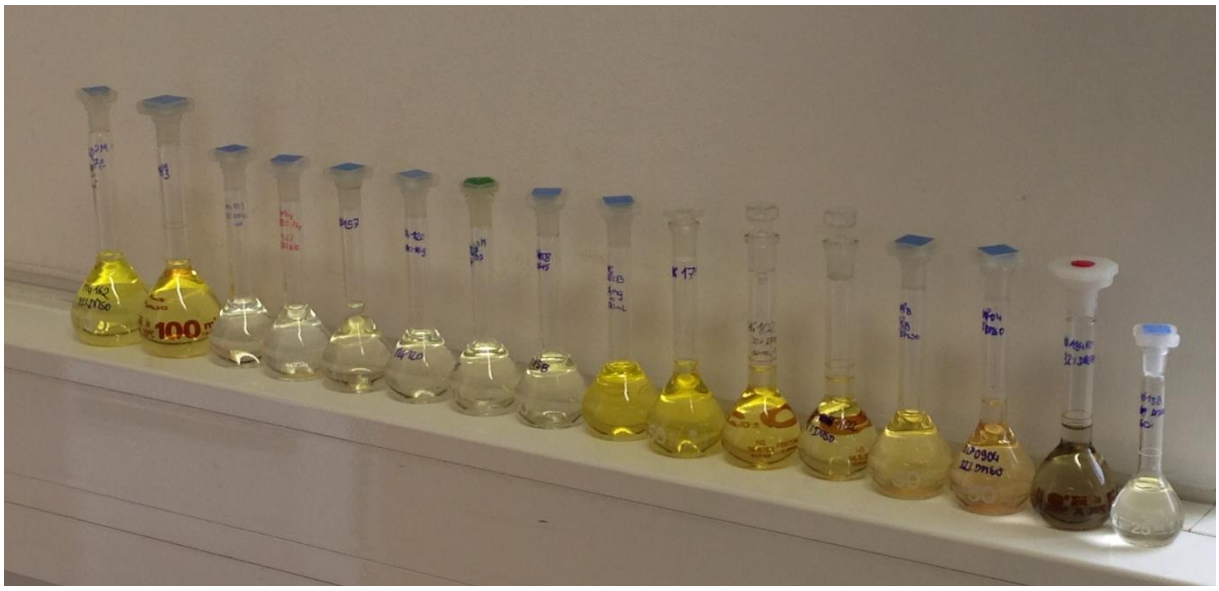


Abb. 8: Stammlösungen der SL-Substanzen (Foto: Sagaischek K.)

Es wurde ohne Lichtschutz gearbeitet, außer bei der Substanz SL4, da diese nach 2 Stunden schon deutliche Zersetzung zeigte. Im zeitlichen Abstand von etwa 70 Minuten war bei SL4 eine deutliche Verfärbung zu erkennen.

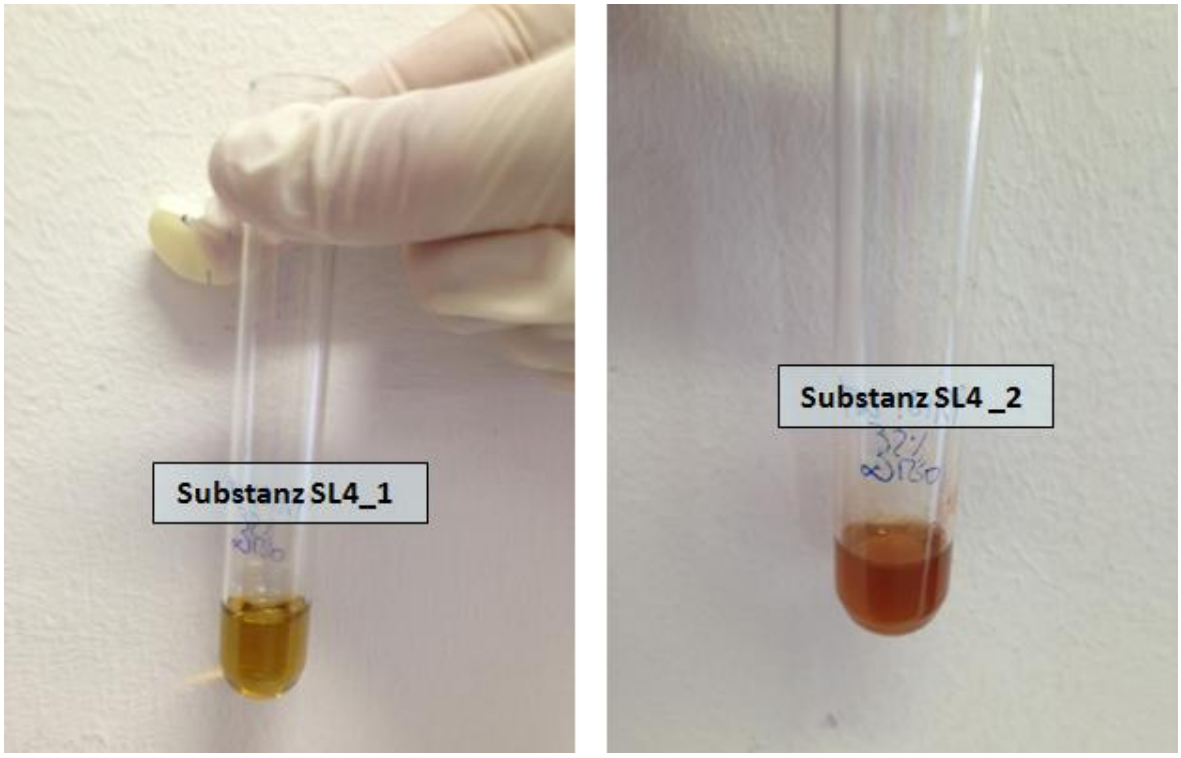


Abb. 9: Verfärbung von SL4 (Fotos: Sagaischek K.)

Schlechtere Löslichkeit oder Verfärbungen schlugen sich auch in qualitativ weniger hochwertigen HPLC- Chromatrogrammen und Ergebnissen nieder.

3.4. Probendurchführung

3.4.1. TestKit System Transil ^{XL}

Die deutsche Firma Sovicell produziert und vertreibt Test Kit-Systeme der Transil^{XL}-Technologie.

Dabei handelt es sich um ein zertifiziertes in Vitro-Testsystem, das aus Mikrotiter-Platten besteht, die speziell für die Plasma-Proteinbindung, intestinale Bindung, mikrosomale Bindung, sowie andere Bindungsmechanismen behandelt werden. Die SL-Substanzen und Doxorubicin.HCl wurden in dieser Arbeit auf die Plasmaprotein-Bindung getestet, während die gleichen Substanzreihe im selben Zeitraum von meiner Kollegin Valerie Klug auf die intestinale und mikrosomale Bindung untersucht wurden.

Die Produktpalette von Plasma-Protein Bindung Transil^{XL} Kits der Firma Sovicell umfasst:

- TRANSIL^{XL} PPB Binding Kit
- TRANSIL^{XL} High Sensitivity Binding Kit
- TRANSIL^{XL} HSA Binding Kit
- TRANSIL^{XL} RSA Binding Kit
- TRANSIL^{XL} AGP (AAG) Binding Kit

Im Rahmen dieser Arbeit wurde mit dem PPB Binding Kit, dem HSA Binding Kit und dem AGP (AAG) Binding Kit gearbeitet.

Das TRANSIL^{XL} PPB Binding Kit erlaubt eine Vorhersage der Bindung von Arzneistoffen und deren Affinität gleichzeitig an HSA und AGP. Der TRANSIL^{XL} HSA Binding Kit dient der Messung der Dissoziationskonstante K_D der Bindung von Arzneistoffen an HSA; während der TRANSIL^{XL} AGP (AAG) Binding Kit diese Dissoziationskonstante für die Bindung an AGP berechnet, um Schwankungen der Plasma-Proteinbindung unter verschiedenen physiologischen Bedingungen und Krankheitszuständen festzustellen. Die Ergebnisse der HSA und AGP Kits können kombiniert werden, um eine genaue Plasma-Proteinbindung zu berechnen und eine Aussage über den freien Anteil eines Arzneistoffes im Plasma machen zu können.

Jedes Binding Kit umfasst eine Mikrotiter-Platte, einen User Guide und einen Decapper.

Die Mikrotiter-Platte besteht aus 12 Spalten von Mikrotiter-Behältnissen, wobei jede Spalte 8 Reaktionskammern, die Wells, beinhaltet. Die Deckel (Caps) jeder Well-Spalte sind zu einem Cap-Band zusammengehängt und gewähren die verlustfreie Entfernung

aller Caps auf einmal. Da die Wells sehr klein sind, ist das Cap-Band sehr praktisch, um eine sichere und saubere Entfernung aller Caps zu garantieren.

Jede Spalte bildet ein Compound und ermöglicht eine Probendurchführung zur Plasma-Protein-Bindung. Eine Platte bietet somit Platz für zwölf Probendurchführungen. Da jede Spalte einzeln aus dem Kit entfernt werden kann, ist eine flexible Probendurchführung von einem bis zu zwölf Durchgängen gewährleistet.

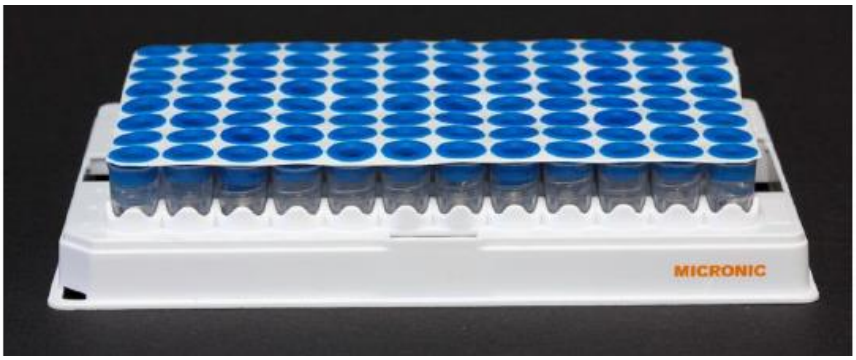


Abb. 10: Transil XL-Mikrotiter Platte (User Guide Screenshot)

Die 8 Wells eines jeden Compounds sind zu ¾ mit PBS Puffer vom pH=7,4 und der Konzentration von 50mM befüllt. Im ersten und letzten Well ist nur dieser Puffer enthalten, da diese zwei Wells den Referenzmessungen dienen (Ref1 & Ref2). In den restlichen Wells (1-6) sind Protein-überzogene Silica-Kugeln enthalten, deren Protein-Konzentration mit zunehmender Well-Nummerierung steigt. Jedes Binding-Kit wird mit einer fixen Konzentration einer Probenlösung inkubiert, während die Konzentration der Proteine in den Wells unterschiedlich ist.

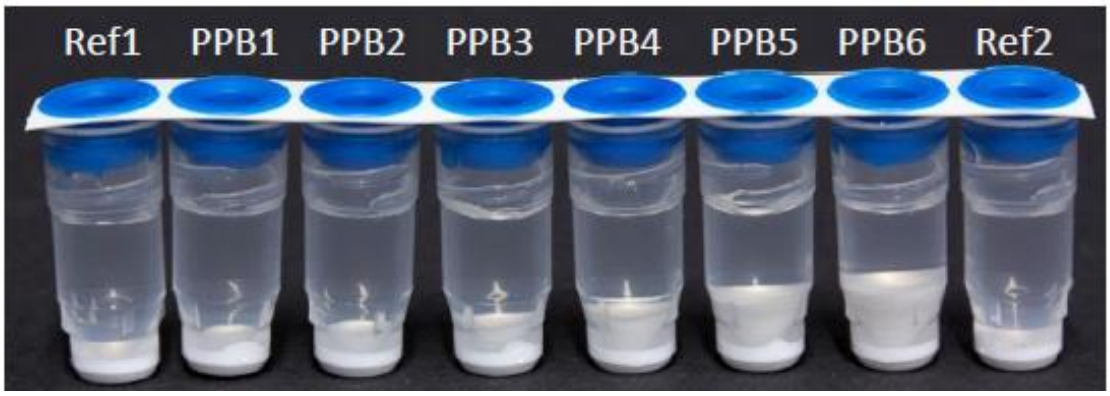


Abb. 11: Transil XL- Compound Reihe, (User Guide Screenshot)

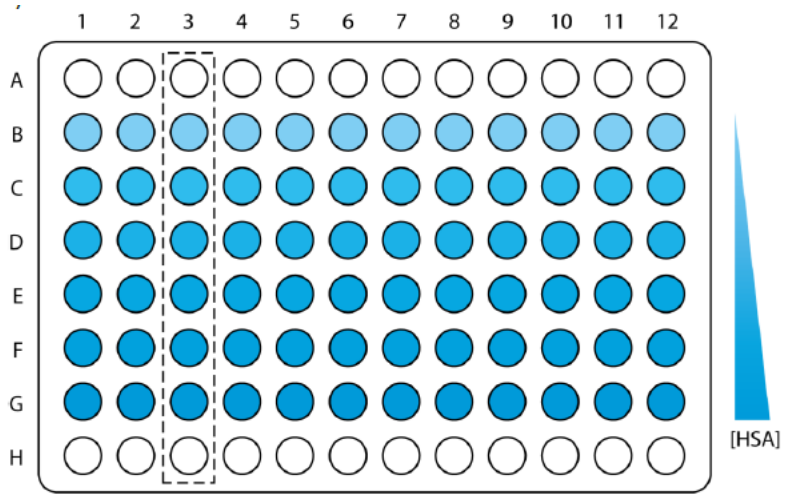


Abb. 12: HSA-Konzentration der Wells schematisch dargestellt (User Guide Screenshot)

Der zum Kit gehörige User Guide wird in einer Ringmappe mitgeliefert und beinhaltet alle Informationen zum Aufbau und zur Durchführung. In der Mappe befindet sich auch eine CD, auf dieser sind der Guide in elektronischer Form, Bei der Software handelt es sich um eine Excel-Arbeitsmappe, in der die Analysendaten der Quantifizierung zur Auswertung eingetragen werden müssen.

Das Material Safety Data Sheet enthält die genaue Bezeichnung des jeweiligen Test-Kits und des Herstellers, sowie zahlreiche Hinweise zur rechtmäßigen Benützung. Es sind auch Anweisungen zur ersten Hilfe gegeben, sollte es zur Aufnahme über die Lunge, oder zum Körperkontakt zwischen Haut oder Augen und den Proteinen des Kits kommen. Die Bestandteile der Kits werden als nicht gefährlich (not hazardous) eingestuft. Trotzdem wird für die Benützung der Kits die Verwendung von Einmal-Handschuhen, Labormantel und Schutzbrille empfohlen.

Die Kits sind mit verschiedenen Analysenverfahren kompatibel, die SL-Substanzen werden mit der HPLC analysiert und die Peakflächen der entstandenen Chromatogramme für die Software zur Auswertung verwendet.

Da es sich bei der Arbeit mit den Kits um Volumina im µl – Bereich handelt, wird mit Kolbenhub-Pipetten und Glas-Einweg-Pipetten gearbeitet.

3.4.2. Transil^{XL} Durchführung

Die Methodik der Test-Kits wurde aus den User-Guides der Fa. Sovicell übernommen und dient als Quelle zur Beschreibung der Durchführung und der Auswertung. [22]

Die nachfolgende Beschreibung der Durchführung gilt sowohl für eine als auch mehrere Compounds der Transil^{XL}-Platten.

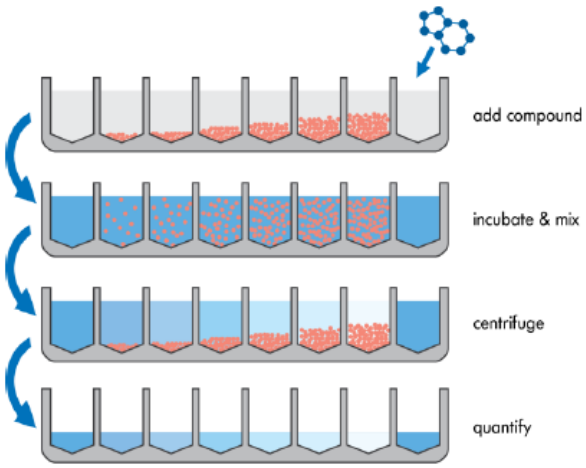


Abb. 13: Arbeitsschritte schematisch dargestellt

- Probenvorbereitung

Bevor mit den Transil^{XL} Platten gearbeitet werden kann, müssen diese aufgetaut und auf Raumtemperatur (20-25°C) gebracht werden, da man die Platten ab dem Zeitpunkt der Lieferung bei -20°C im Eis aufbewahrt. Sie werden entweder am Vorabend in den Kühlschrank bei 4°C gestellt oder 3 Stunden vor Arbeitsbeginn bei Raumtemperatur stehen gelassen.

Unmittelbar vor Arbeitsbeginn werden die Kits 5 Sekunden lang bei 750 g zentrifugiert, da sich in den Wells eine Proteinsuspension befindet, und diese am Boden gesammelt sein sollte. Wichtig ist, dass die Wells fest mit dem Cap-Band verschlossen sind.

- Herstellung der Probenlösung

Um die richtige Endkonzentration der Probenlösung in den Wells zu erreichen, wird pro zu analysierender Substanz eine 80µM Stammlösung mit 32% DMSO als Lösungsmittel hergestellt. Die Endkonzentration von DMSO in den Wells soll 2-6% betragen, deswegen wird eine 16fach konzentrierte DMSO Lösung bereitet (→32%), da durch den bereits vorhandenen PBS Puffer in den Wells das Lösungsmittel verdünnt wird. Die Stammlösung muss eine Konzentration von 80µmol aufweisen, da die Endkonzentration in den Wells 5µmol betragen soll. Auch hier wird die 16fach konzentrierte Stammlösung bereitet.

Wichtig ist, dass sich die Konzentrationen in den angegebenen Bereichen befinden, da sonst keine zufriedenstellende Quantifizierung gewährleistet werden kann.

- Probendurchführung

Vor Probenzugabe werden die Caps der Wells mit dem Decapper entfernt. Dieser garantiert eine sichere und protein-verlustfreie Öffnung der Wells.

- Probenzugabe

Die Stammlösung der Probe wird auf dem Vortexer durchgeschüttelt und 15µl mittels Kolbenhubpipetten in jedes Well transferiert. Dabei wird chronologisch von Ref1 nach Ref2 vorgegangen und vor jeder neuen Probenzugabe eine neue, saubere Pipettenspitze aufgesetzt. Die Wells von Ref1 und Ref2 ohne Protein dienen der Ermittlung von unspezifischen Bindungen.

Besonders zu beachten ist, dass mit der Pipette nicht in die abgesetzte Proteinmenge gestochen und irrtümlich etwas davon aufsaugt wird. Nach Pipettieren der Probenlösung werden die Wells mit dem Cap-Band wieder gut verschlossen.

Abb. 14: Arbeitsplatz: Zugabe der Probenlösung mit Kolbenhub-Pipette (Foto: Sagaischek K.)

- Inkubation

Um die Platten zu inkubieren werden diese auf den plate shaker, einen auch für Transil^{XL}-Platten passenden Schüttler, gestellt. In diesem Punkt unterscheiden sich die Arbeitsschritte der einzelnen Transil^{XL}-Kits.

Die HSA- und PPB-Transil^{XL} Platten werden auf dem plate shaker 12 min lang bei 1000 rpm inkubiert, während die AGP- Transil^{XL} Kits nur für 30 sec bei 1000 rpm auf dem plate shaker geschüttelt werden. Die AGP Kits werden danach noch 2 Minuten mit Boden nach oben („bottom-up“) sachte in der Hand geschüttelt.

Während der Inkubation am Schüttler und in der Hand vollzieht sich die Proteinbindung.

- Phasentrennung von Proteinsediment und Pufferlösung

Unabhängig von Art des Kits wird dieses nach der Inkubation erneut bei 750g, diesmal für 10 Minuten, zentrifugiert, damit die Proteinmenge sedimentiert und der entstandene Überstand für die weitere Durchführung klar und partikelfrei ist. Die Phasentrennung ist bei diesem Schritt sehr wichtig, damit beim nachfolgenden Arbeitsschritt eine Verunreinigung verhindert werden kann.

- Entnahme des Überstandes

Ist der Überstand nach dem Zentrifugieren klar und schwebstofffrei, müssen 50-100 µl davon entnommen werden, um damit die Proteinbindung zu analysieren. Wichtig ist, dass nur der klare Überstand entnommen wird, und keine Festsubstanz mitaufgesaugt wird.

Zur Analyse der Proteinbindung wurden etwa 70 µl des Überstandes mit einer Glas-Einwegpipette und Gummisauger entnommen und in einen Mikroeinsatz (Vial) für HPLC-Braunglas Fläschchen transferiert. Auch die Glas-Einwegpipette muss nach jeder Überführung eines Überstandes verworfen und eine neue für das nächste Well benutzt werden. Hier ist zu beachten, dass man luftblasenfrei arbeitet, da Luftblasen die HPLC-Apparatur beschädigen können, wenn diese vom Gerät aufgesaugt werden.

Abb. 15: Arbeitsplatz: Überführen des Überstands in Vials der HPLC-Braunglas Fläschchen mit Glaseinweg-Pipetten

3.4.3. HPLC-Analyse

Die Analyse der Proben erfolgte bei den SL-Substanzen und Doxorubicin.HCl mit der HPLC. Die nachfolgende Tabelle repräsentiert die HPLC-Daten aller Proben. Angeführt sind die benutzen HPLC-Säulen, wobei die SL-Substanzen mit der ACE Säule oder der Nukleosil Säule analysiert wurden, und Doxorubicin.HCl mit der LiChrospher 100 Rp 18 ec Säule.

Die SL-Substanzen wurden bei einer Wellenlänge von 270nm mit dem UV-Detektor detektiert, Doxorubicin.HCl hingegen bei 485nm. Die unterschiedlichen Retentionszeiten, die charakteristische Parameter für jede einzelne Substanz sind, und die jeweiligen Eluenten werden auch in der Tabelle aufgeführt.

Substanz	**HPLC – Säule**	**Wellenlänge [nm]**	**Retentionszeit [min]**	**Eluent**
SL 1	ACE	270	5,2	Isokratisch 1
SL 2	ACE	270	5,6	Isokratisch 1
SL 3	ACE	270	5,6	Isokratisch 1
SL 4	ACE	270	3,1	Isokratisch 2
SL 5	ACE	270	7,2	Isokratisch 2
SL 6	ACE	270	4,5	Isokratisch 1
SL 7	ACE	270	5,6	Isokratisch 1
SL 8	ACE	270	4,3	Isokratisch 1
SL 9	Nukleosil	270	7,4	Isokratisch 1
SL 10	ACE, Nukleosil	270	7,5 / 10,5	Isokratisch 1
SL 11	ACE, Nukleosil	270	4,7	Isokratisch 1
SL 12	ACE	270	7,9	Isokratisch 1
SL 13	ACE	270	5,3	Isokratisch 1
SL 14	ACE	270	4,6	Isokratisch 1
Doxorubicin.HCl.	LiChrospher 100 Rp 18 ec	485	3,2	Eluent für Doxorubicin.HCl

Tab. 6: HPLC-Daten der Proben

4. Auswertung und Ergebnisse

4.1. Transil^{XL} Software

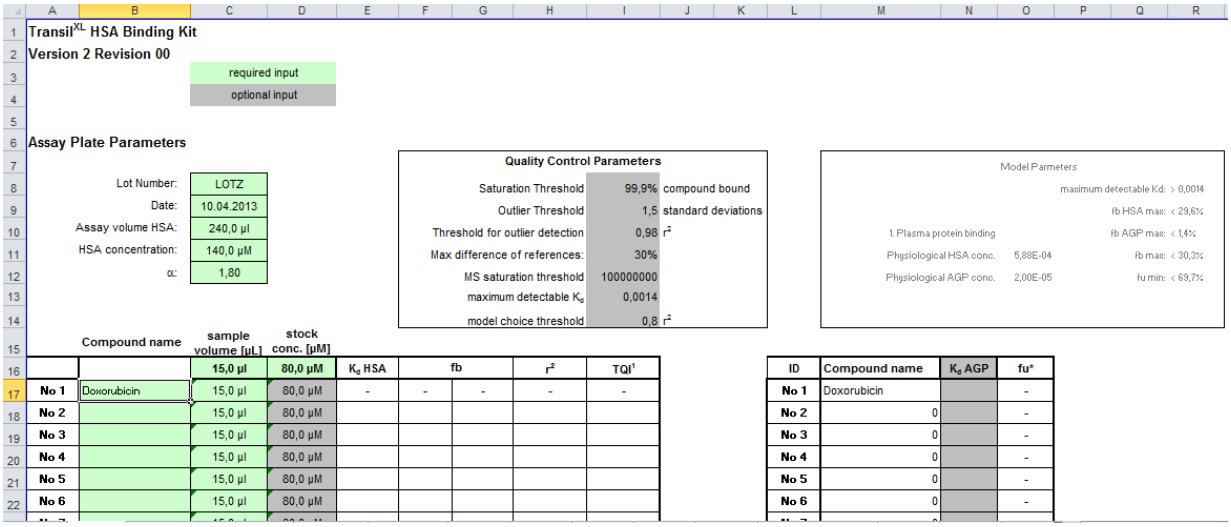
Die Auswertung der HPLC Analyse der Transil^{XL} Binding Kits wurde mit einer Microsoft Excel Arbeitsmappe durchgeführt, die eigens für das Kit mit allen Berechnungen versehen wurde. Die Transil^{XL} User Guides als Anleitung zur Durchführung sind Ringmappen, die auch eine CD mit sich führen, auf welcher sich der Guide in elektronischer Form und die Excel Datei zu Auswertung befinden.

4.1.1. Microsoft Excel Auswertung am Beispiel HSA von Doxorubicin.HCl

In der Microsoft Excel Datei ist die **ersten Seite** eine Übersichtsseite, die *main page*. Auf dieser sind die wichtigsten Analysenparameter zusammengefasst.

Um die Datei anfangs zu aktivieren, müssen alle grün-unterlegten Felder ausgefüllt werden. Die erste grün-unterlegte Spalte der Assay Plate Parameters verlangt Daten aus einem Zertifikat. Die benötigten Parameter setzten sich aus der Lot Number, dem Prüf-Datum, dem HSA-Proben-Volumen, der HSA Konzentration und einem griechischem Buchstaben, für den eine Zahl steht, zusammen. Die Parameter sind spezifisch für jeden Kit (HSA, AGP, PPB) und werden aus dem beiliegendem Zertifikat entnommen. Ohne die Zertifikats-Daten ist keine Auswertung der Ergebnisse möglich.

Die grau unterlegten Felder benötigten Daten aus Excel-sheets anderer Binding Kits, da AGP und HSA kombiniert werden können.



Transil^{XL} HSA Binding Kit
Version 2 Revision 00

required input
optional input

Assay Plate Parameters

Lot Number:	LOTZ
Date:	10.04.2013
Assay volume HSA:	240,0 µl
HSA concentration:	140,0 µM
α:	1,80

Quality Control Parameters		
Saturation Threshold	99,9%	compound bound
Outlier Threshold	1,5	standard deviations
Threshold for outlier detection	0,98	r^2
Max difference of references:	30%	
MS saturation threshold	100000000	
maximum detectable K_d	0,0014	
model choice threshold	0,8	r^2

Model Parmeters		
		maximum detectable Kd: > 0,0014
		fb HSA max: < 29,6%
1. Plasma protein binding		fb AGP max: < 1,4%
Physiological HSA conc.	5,88E-04	fb max: < 30,3%
Physiological AGP conc.	2,00E-05	fu min: < 69,7%

	Compound name	sample volume [µL]	stock conc. [µM]	K_d HSA	fb		r^2	TQI[1]
		15,0 µl	80,0 µM					
No 1	Doxorubicin	15,0 µl	80,0 µM	-	-	-	-	-
No 2		15,0 µl	80,0 µM					
No 3		15,0 µl	80,0 µM					
No 4		15,0 µl	80,0 µM					
No 5		15,0 µl	80,0 µM					
No 6		15,0 µl	80,0 µM					

ID	Compound name	K_d AGP	fu*
No 1	Doxorubicin		-
No 2	0		-
No 3	0		-
No 4	0		-
No 5	0		-
No 6	0		-

Abb. 16: main page (Microsoft Excel Screenshot)

Die auf der gesamten main page ersichtlichen, für die Proben relevanten, Parameter sind:

- compound name

Der compound name ist die Bezeichnung der Probe, angefangen von Doxorubicin.HCl bis SL 14. Diesen muss man auf der main page eintragen, in Summe 15mal.

- sample volume [µl]

Das sample volume in µl bezeichnet das Proben-Volumen, das immer 15 µl beträgt; genau das Volumen der Stammlösung, welches in die Wells pipettiert wird.

- stock conc. [µM]

Die stock concentration in µM bezeichnet die Konzentration der Stammlösung der Probe, das sind 80µM.

- K_D HSA

Die Dissoziationskonstante K_D, auch Affinitätskonstante, ist das Maß für eine Dissoziationsreaktion, wie auch die Proteinbindung eine ist, und wird zur Berechnung der Proteinbindung benötigt.

- f_b

Die fraction bound ist der gebundene Anteil des Arzneistoffes an, in diesem Fall, HSA. Er wird in Prozent mit ±Grenzwerten angegeben und wird aus den K_D-Werten berechnet.

- r^2

r^2 beschreibt den Korrelationskoeffizienten und ist ein Maß für die Linearität der Messwerte.

- TQI

Der TQI, auch Transil^{XL} Quality Index, ist ein Qualitätsindex, der die Güte der Auswertung beschreibt. Dabei gibt 10 die höchste und 0 die niedrigste Qualität dieser Analyse an. Der TQI wird zusätzlich optisch durch einen farbigen Punkt ergänzt, wobei grün für einen TQI von 7-10, gelb für 5-7, und rot für einen TQI <5 steht.

- K_D AGP

Die Transil^{XL} Binding Kits von HSA und AGP müssen für die Berechnung des jeweiligen freien Anteils kombiniert werden. Deswegen sind die AGP-K_D Werte der gleichen Substanzen unerlässlich für die Vollständigkeit der Berechnung, eine HSA-Kit alleine reicht nicht für eine vollständige Aussage des Bindungsprofils eines Arzneistoffes an die Plasmaproteine aus. In das Excel sheet des HSA Binding Kits werden in die zweite Tabelle

die K_D-Werte der AGP Bindung eingetragen, um auch den ungebundenen Anteil der Substanz an HSA zu berechnen.

- f_u

Die fraction unbound ist der freie oder ungebundene Anteil des Arzneistoffes im Plasma. Zur Berechnung der f_u von HSA benötigt man, wie bereits erwähnt, den K_D AGP der gleichen Substanz. Der freie Anteil eines Arzneistoffes ist der, der eine pharmakologische Wirkung erzielen kann. Die K_D-Werte von HSA und AGP waren auch hilfreich für die Binding Kits meiner Kollegin, da diese bei ihrer Arbeit mit dem Transil^{XL}- Intestinal Binding Kit notwendig zur Berechnung des scheinbaren Verteilungsvolumens V_D waren.

- Model Parameters

Die Model Parameters geben die Konzentration der jeweiligen Proteine und die Bindungskapazität des Kits an.

Die **zweite Seite** der Excel Datei ist die *raw data page*.

Sie besteht aus leeren Tabellen, in der die Ergebnisse des Analysenverfahrens eintragen werden. Da unser Analysenverfahren der Wahl die HPLC ist, werden als raw data die Flächen der Substanzpeaks in die Spalte „area/height“ eingetragen.

Die Bezeichnung der Probe, die als compound name in der ersten Seite eingetragen wurde, wird auf der raw data page übernommen. So ist eine Zuordnung der areas zu den Proben leicht möglich.

B107 | fx | 0

	A	B	C	D	E	F	G	H	I
1	Please enter the peak area or concentration data in column G below								
2									
3		Please leave missing data fields blank							
4									
5	test article				Well	Sample	Area / height	nm / amu	Note
6	Doxorubicin				A-1	Ref 1			
7	Doxorubicin				B-1	Well 1			
8	Doxorubicin				C-1	Well 2			
9	Doxorubicin				D-1	Well 3			
10	Doxorubicin				E-1	Well 4			
11	Doxorubicin				F-1	Well 5			
12	Doxorubicin				G-1	Well 6			
13	Doxorubicin				H-1	Ref 2			
14	cmp2				A-2	Ref 1			
15	cmp2				B-2	Well 1			
16	cmp2				C-2	Well 2			
17	cmp2				D-2	Well 3			
18	cmp2				E-2	Well 4			
19	cmp2				F-2	Well 5			
20	cmp2				G-2	Well 6			
21	cmp2				H-2	Ref 2			
22	cmp3				A-3	Ref 1			

Abb. 17: raw data page – leer (Microsoft Excel Screenshot)

Die HPLC lieferte oft Chromatogramme mit unterschiedlich vielen Peaks. Eigentlich sollte bei reinen Proben, wie Doxorubicn.HCl, nur ein Substanzpeak im Chromatogramm ersichtlich sein. Da die SL-Proben aber von unbekannter Löslichkeit, Beständigkeit und Reinheit waren, waren bei diesen Chromatogrammen oftmals mehrere Peaks zu erkennen. In so einem Fall muss der als Substanzpeak erkennbare Peak durch händisches Nachintegrieren im HPLC-Computerprogramm korrigiert werden, um ein verwertbares Ergebnis zu bekommen. Bei zusammenhängenden Peaks wird die Area über alle Peaks berechnet, da diese sich eine Basislinie teilen. Beim händischen Nachintegrieren erhält der Substanzpeak eine eigene Basislinie und dadurch auch eine andere Area.

Bei Doxorubicin.HCl war dies nicht notwendig, die Fläche des jeweiligen Peaks wurde ohne Nachbearbeitung in die Tabelle der raw data page übertragen.

Die Wertigkeit der Zahlenbeträge der Areas sollte idealerweise von Well 1 bis Well 6 abnehmen, da die Proteinmenge von Well zu Well steigt und so immer mehr Arzneistoff vom Protein gebunden werden kann. Die Referenzwerte in Ref1 und Ref2 sollten jeweils höher als die anderen Werte sein.

Die raw data Werte aller Proben sind in Tabellen im Anhang (s. 12.1.) zusammengefasst.

N16

Please enter the peak area or concentration data in column G below

Please leave missing data fields blank

test article	Well	Sample	Area / height	nm / amu	Note
Doxorubicin	A-1	Ref 1	284,700		
Doxorubicin	B-1	Well 1	276,900		
Doxorubicin	C-1	Well 2	264,500		
Doxorubicin	D-1	Well 3	240,000		
Doxorubicin	E-1	Well 4	258,700		
Doxorubicin	F-1	Well 5	242,900		
Doxorubicin	G-1	Well 6	201,900		
Doxorubicin	H-1	Ref 2	281,900		
cmp2	A-2	Ref 1			
cmp2	B-2	Well 1			
cmp2	C-2	Well 2			
cmp2	D-2	Well 3			
cmp2	E-2	Well 4			
cmp2	F-2	Well 5			
cmp2	G-2	Well 6			
cmp2	H-2	Ref 2			
cmp3	A-3	Ref 1			

Abb. 18: raw data page - Doxorubicin.HCl mit Analysenergebnissen (Microsoft Excel Screenshot)

Die **restlichen Arbeitsblätter** der Excel-Datei dienen der Detailaufschlüsselung der Auswertung.

- AGP und HSA Proteinbindung

Das Excel-Programm berechnet die Proteinbindung und die Qualitätsparameter unmittelbar nach Eingabe der Zertifizierungs-Parameter, der compound Bezeichnung, den Konzentrationsangaben und den raw data der Analyse.

Die Plasmaprotein Bindung wird in Bezug auf die Dissoziationskonstante, auch Affinitätskonstante, K_D gemessen:

$$KD = \frac{[A] \times [P]}{[AP]} \qquad (1)$$

A ist die Konzentration des Arzneistoffes gebunden an ein Protein P, wobei A die freie Konzentration des Arzneistoffes und P die freie Konzentration des Proteins kennzeichnet.

Die freie Konzentration eines Arzneistoffes kann auch so ausgedrückt werden:

$$[A] = fu \times ([A] + [AP]) \qquad (2)$$

Durch Einsetzen von Formel (2) in Formel (1) und Umformen wird ein lineares Modell erstellt, das zur Berechnung der Werte aus den Kits passt und eine Steigungsmaß von $1/K_D$ und einen Achsenabschnitt von 0 hat:

$$\frac{fb}{fu} = \frac{1}{KD} \times P \qquad (3)$$

Die Konzentration des Protein-Arzneistoff-Komplexes $[AP]$ muss dabei viel kleiner als die Gesamt-Konzentration in jedem Well sein.

Der totale gebundene Anteil (f_b, fraction bound) kann durch die K_D Werte von AGP für HSA vorhergesagt werden, auch die physiologische Konzentration dieser Plasma-Proteine im menschlichen Blut.

$$fb = 1 - \frac{1}{1 + \frac{[HSA]}{KD\ (HSA)} + \frac{[AGP]}{KD\ (AGP)}} \qquad (4)$$

Die Berechnung durch die Excel-Datei dient der Beurteilung des gebundenen Anteils von Arzneistoffen, allerdings nur an die Plasma-Proteine HSA und AGP. Plasma-Proteine, die in

geringerer Konzentration vorkommen, wie Lipoproteine, Transcortin oder Sexualhormone, werden hier nicht berücksichtigt.

In der Detailaufschlüsselung der Excel-Arbeitsblätter sind in Tabellen und Graphen alle Ergebnisse genau aufgelistet.

Ganz oben auf der Seite befindet sich eine Tabelle mit der Bewertung der Analysenergebnisse. Hier ist die Qualität der Messwerte ersichtlich. Diese werden mit *ok*, *inconsistent* (widersprüchlich) oder *outlier* (Ausreißer) bewertet, wobei ein outlier nicht mehr in die Berechnung miteinbezogen wird.

K51

	A	B	C	D	E	F	G	H	I	J	K
1			test article No		1	Doxorubicin					
2											
3											
4			5,000 µM					0 nm / amu			
5	adj. Ref 1		284,700	A		av ref ok					
6	Ref 2		281,900	ref diff ok		max ref ok					
7	Signal Reference:		283,300	283,300	283,300	283,300	283,300	283,300			
8	TRANSIL well:		I	II	III	IV	V	VI			
9	Signal in TRANSIL wells:		276,900	264,500	240,000	258,700	242,900	201,900	ok		
10											
11			-								
12			missing values								
13			1	1	1	1	1	1			
14			outlier detection								
15	consistency		ok	ok	ok	ok	ok	ok			
16	saturation		ok	ok	ok	ok	ok	ok			
17	statistics		ok	ok	outlier	ok	ok	ok			
18											
19								r^2= 0,95271			
20	QC index:	9,4	k_D= 3,73E-04		fb=	61,2% ± 3,673%					
21	warning messages:										
22											

Abb. 19: Detailansicht Doxorubicin.HCl, erste Tabelle (Microsoft Excel Screenshot)

Gleich unter der ersten Tabelle, in blau gehalten, sind der TQI, der K_D-Wert, f_b und r^2 ersichtlich.

Der **TQI** stellt sich aus, wie bereits erwähnt, 5 Parametern zusammen:

- Model fit

Die Proteinbindung wird gemäß Formel (3) berechnet. Mit sehr guten Messwerten würde sich ein Steigungsmaß ergeben, das genau der Affinitätskonstante K_D entsprechen würde, und einen Achsenabschnitt (intercept) von 0. Ab einem intercept außerhalb der Grenzen [-0.5, 0.5] und innerhalb der Grenzen [-1.5, 1.5] sinkt der TQI. Sollte der intercept auch außerhalb der zweiten Grenzen liegen, ist der Teilbetrag dieser TQI Bewertung gleich 0. So ist der intercept gleichzeitig auch ein Kontrollparameter.

Da ein idealer Model fit kaum erzielt werden kann, dient das Diagramm der K_D-Estimation (Abb. 20, Diagramm 2) der näheren Erläuterung. Der Model fit hat großen Einfluss auf den TQI-Wert.

- Die Übereinstimmung der gemessen und vorhergesagten Referenz-Signale

Bei der Bestimmung der Affinitätskonstanten über fünf oder sechs verschiedene Proteinkonzentrationen mit zwei Bezugs-Referenz-Wells ohne Protein, kann eine erwartete Peakfläche durch lineare Regression der Messwerte vorhergesagt werden. Im Diagramm des Reference check (Abb. 20, Diagramm 3) wird der Kehrwert der Peakflächen gegen den Proteingehalt aufgetragen. Auf der so entstehenden Linearen, die durch die zwei Referenzwerte gegeben ist, sollten alle Messwerte der proteinhaltigen Wells liegen. Ist ein Referenzwert ein Ausreißer, weil sein Wert geringer als die Messwerte der Wells 1-6 ist, wird stattdessen der Wert der anderen Referenz zweimal verwertet. Eine Abweichung von der Linearen kann durch ein schlechtes Bindungsvermögen, resultierend aus verminderter Löslichkeit oder Unbeständigkeit, entstehen.

- Der Korrelationskoeffizient

Der Korrelationskoeffizient als Maß für den linearen Zusammenhang, berechnet aus oben beschriebener Gleichung (3), wird auch zur Bewertung des TQI herangezogen.

- Die Zahl der Ausreißer, bzw. der fehlenden Messwerte
- Die Kontinuität der Messwerte

Durch die steigende Proteinkonzentration in den Wells sollte auch die Proteinbindung stetig steigen. Bei einem Ausreißer wird die Messung als inconsistent (widersprüchlich) bezeichnet, sollte dieser für zwei aufeinanderfolgende Wells gelten, wird ein Messwert von der Berechnung ausgeschlossen und gilt als outlier (Ausreißer).

Das erste der drei nachfolgenden Diagramme zeigt die Höhe der Signale der Messwerte als blaue Balken, wobei die rote Linie darüber die Referenzen darstellt. Die Einheiten der y-Achse entsprechen den Einheiten der raw data page.

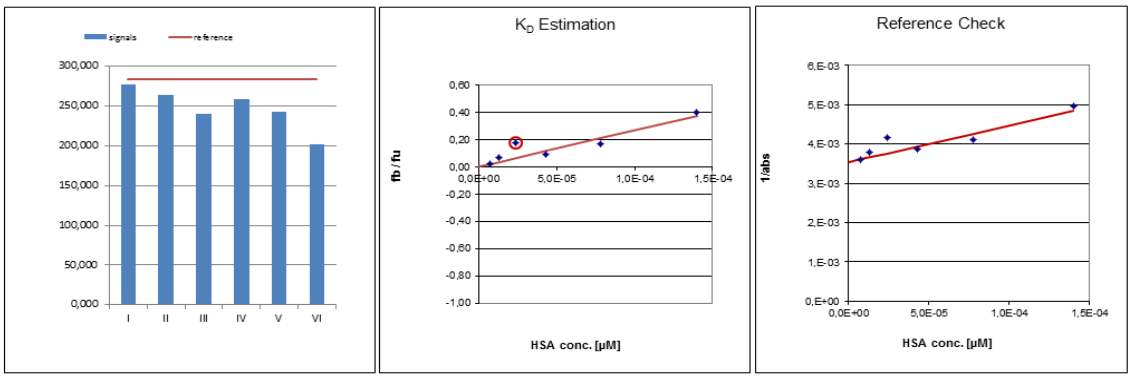
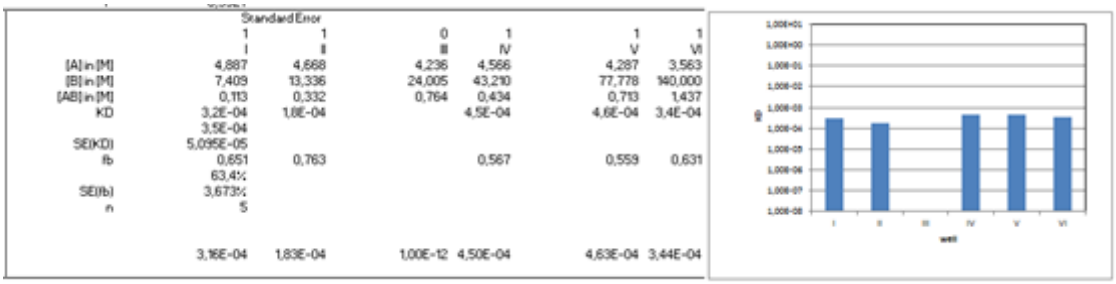


Abb. 20: Signalhöhe der Messwerte(1), K_D-Estimation(2) und Reference Check(3) (Microsoft Excel Screenshot)

Die Affinitätskonstante $\mathbf{K_D}$ und der gebundene Anteil $\mathbf{f_b}$ werden durch die oben beschriebene Formeln berechnet. Der Korrelationskoeffizient $\mathbf{r^2}$ wird als Maß für die Linearität der Messungen angegeben.

Die letzte Tabelle der Detailaufschlüsselung beinhaltet ein Diagramm, in dem die K_D-Werte der einzelnen Wells (1-6) aufgeführt werden.



	StandardError					
	1	1	0	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,887	4,668	4,236	4,566	4,287	3,563
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,113	0,332	0,764	0,434	0,713	1,437
KD	3,2E-04	1,8E-04		4,5E-04	4,6E-04	3,4E-04
	3,5E-04					
SE(KD)	5,095E-05					
fb	0,651	0,763		0,567	0,559	0,631
	63,4%					
SE(fb)	3,673%					
n	5					
	3,16E-04	1,83E-04	1,00E-12	4,50E-04	4,63E-04	3,44E-04

Abb. 21: Letzte Tabelle der Detail-Aufschlüsselung - K_D Werte (Microsoft Excel Screenshot)

Auch ersichtlich in der Detailaufschlüsselung ist die Konzentration der Proteine in den Wells. In der nachfolgenden Tabelle ist die Zunahme der Protein-Konzentration, angegeben in Mol/l, übersichtlich dargestellt.

Well	**Protein Konzentration [M]**		
	HSA binding Kit	**AGP Binding Kit**	**PPB Binding Kit**
Ref1	-	-	-
1	0,0000074	0,0000040	0,0000074
2	0,000013	0,0000059	0,000013
3	0,000025	0,0000089	0,000025
4	0,000043	0,000013	0,000043
5	0,000078	0,000020	0,000078
6	0,00014	0,000030	0,00014
Ref2	-	-	-

Tab. 7: Protein-Konzentrationen in den Kits

Die restlichen Tabellen der Detailaufschlüsselung zeigen die genauen Ergebnisse der einzelnen Wells, auf der main page sind dann die Ergebnisse für das ganze Compound angegeben.

4.2. HSA – Auswertung

Transil^{XL} HSA Binding Kit

Version 2 Revision 00

required input
optional input

Assay Plate Parameters

Lot Number:	LOTZ
Date:	10.04.2013
Assay volume HSA:	240,0 µl
HSA concentration:	140,0 µM
α:	1,80

Quality Control Parameters		
Saturation Threshold	99,9%	compound bound
Outlier Threshold	1,5	standard deviations
Threshold for outlier detection	0,98	r^2
Max difference of references:	30%	
MS saturation threshold	100000000	
maximum detectable K_d	0,0014	
model choice threshold	0,8	r^2

Model Parmeters			
		maximum detectable Kd:	> 0,0014
		fb HSA max:	< 29,6%
1. Plasma protein binding		fb AGP max:	< 1,4%
Physiological HSA conc.	5,88E-04	fb max:	< 30,3%
Physiological AGP conc.	2,00E-05	fu min:	< 69,7%

	Compound name	sample volume [µL]	stock conc. [µM]					
		15,0 µl	80,0 µM	K_d HSA	fb		r^2	TQI[1]
No 1	Doxorubicin	15,0 µl	80,0 µM	3,73E-04	61,2%	± 0,04	0,9527	9,4
No 2	SL 5	15,0 µl	80,0 µM	NaN	-	-	-	-
No 3	SL 4	15,0 µl	80,0 µM	9,10E-06	98,5%	± 0,00	0,9326	7,4
No 4	SL 6	15,0 µl	80,0 µM	4,25E-05	93,3%	± 0,01	0,9816	9,4
No 5	SL 7	15,0 µl	80,0 µM	3,65E-05	94,2%	± 0,01	0,9948	9,6
No 6	SL 14	15,0 µl	80,0 µM	3,19E-05	94,9%	± 0,00	0,9987	8,1
No 7	SL 1	15,0 µl	80,0 µM	4,57E-05	92,8%	± 0,17	0,9812	8,1
No 8	SL 8	15,0 µl	80,0 µM	3,14E-05	94,9%	± 0,01	0,9850	8,2
No 9	SL 12	15,0 µl	80,0 µM	9,24E-06	98,5%	± 0,05	0,9948	7,1
No 10	SL 9	15,0 µl	80,0 µM	7,41E-05	88,8%	± 0,01	0,9801	9,5
No 11	SL 2	15,0 µl	80,0 µM	4,11E-05	93,5%	± 0,01	0,9961	9,6
No 12	SL 3	15,0 µl	80,0 µM	6,57E-05	90,0%	± 0,06	0,9382	7,2
No 13	SL 13	15,0 µl	80,0 µM	1,05E-06	99,8%	± 0,00	0,9833	7,4
No 14	SL 11	15,0 µl	80,0 µM	6,26E-05	90,4%	± 0,02	0,9680	8,2
No 15	SL 10	15,0 µl	80,0 µM	1,51E-05	97,5%	± 0,00	0,9781	6,9

ID	Compound name	K_d AGP	fu*
No 1	Doxorubicin	4,05E-04	38,1%
No 2	SL 5	1,40E-03	-
No 3	SL 4	1,40E-03	< 1,5%
No 4	SL 6	1,60E-04	6,7%
No 5	SL 7	1,20E-06	3,0%
No 6	SL 14	3,21E-06	3,9%
No 7	SL 1	2,59E-04	7,2%
No 8	SL 8	5,06E-06	4,2%
No 9	SL 12	2,46E-05	1,5%
No 10	SL 9	3,63E-06	6,9%
No 11	SL 2	7,51E-05	6,4%
No 12	SL 3	2,51E-05	9,3%
No 13	SL 13	3,15E-05	0,2%
No 14	SL 11	8,75E-05	9,4%
No 15	SL 10	4,07E-06	2,2%

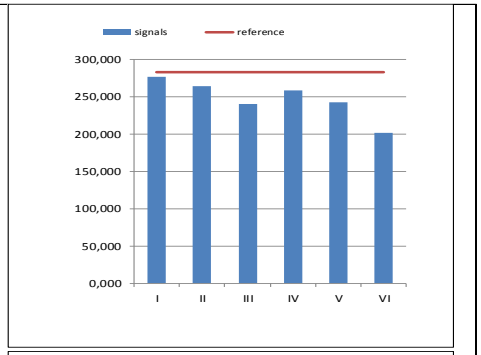
	test article No	1		**Doxorubicin**			
	5,000 µM					0 nm / amu	
adj. Ref 1	284,700	A		av ref ok			
Ref 2	281,900	ref diff ok		max ref ok			
Signal Reference:	283,300	283,300	283,300	283,300	283,300	283,300	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	276,900	264,500	240,000	258,700	242,900	201,900	ok

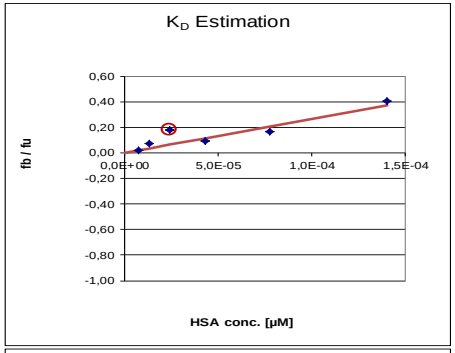
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	**outlier**	ok	ok	ok

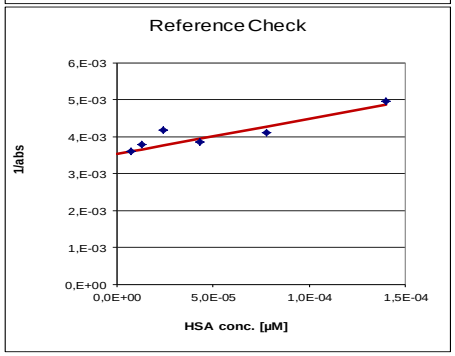
QC index: 9,4 **k_D= 3,73E-04** **fb= 61,2% ± 3,673%** **r^2= 0,95271**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,61E-03	3,78E-03	4,17E-03	3,87E-03	4,12E-03	4,95E-03
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		2,3%	6,6%	15,3%	8,7%	14,3%	28,7%
(1-fu)/fu		0,023	0,071	0,180	0,095	0,166	0,403
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,023	0,071	0,180	0,095	0,166	0,403
slope		2438,48101					
intercept		0,03228					
r^2		0,8386					
prediction: (1-fu)/fu		5,0E-02	6,5E-02	9,1E-02	1,4E-01	2,2E-01	3,7E-01
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,023	0,071	0,180	0,095	0,166	0,403
slope		2438,48101					
intercept		0,03228					
r^2		0,8386					
prediction		0,050	0,065	0,091	0,138	0,222	0,374
residuals		-0,027	0,006	0,090	-0,043	-0,056	0,030
standarized residuals		0,000	0,116	1,659	0,000	0,000	0,546
Threshold		1,5					
statistical outlier	5	1	1	0	1	1	1
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
		0,023	0,071		0,095	0,166	0,403
slope		2682,52457	**3,73E-04**				
intercept		0,00060					
r^2		0,9527					
prediction	0,001	0,020	0,036	0,065	0,117	0,209	0,376
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	0,180	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,11	0,33	0,76	0,43	0,71	1,44
free protein con. [µM]		7,30	13,00	23,24	42,78	77,06	138,56
free protein con. [M]	0	7,3E-06	1,3E-05	2,3E-05	4,3E-05	7,7E-05	1,4E-04
binding sites covered [%]		1,5%	2,5%	3,2%	1,0%	0,9%	1,0%
warning?							







reg	**3,73E-04**	**0,95271**	
avg	**3,51E-04**		1
choice	**3,73E-04**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,61E-03	3,78E-03	4,17E-03	3,87E-03	4,12E-03	4,95E-03
valid albumin conc. [µM]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
valid 1/signal		3,6E-03	3,8E-03		3,9E-03	4,1E-03	5,0E-03
slope		9,46885					
intercept		0,00353					
r^2		0,9527					
predicted 1/signal	0	3,6E-03	3,7E-03	3,8E-03	3,9E-03	4,3E-03	4,9E-03
Predicted Reference Value		283,13					
Actual Reference Value		283,30					
Relative deviation		0,1%					

QC index: 9,4

no. data points	9	reference	10	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%	10	0,9999		A	10	6	10	6	10	data points	
6		20,0%	9	0,999		M		5		5		reference	
5	9	50,0%	8	0,99		R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		0,00	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	-0,027	0,006	0,090	-0,043	-0,056	0,030
	0,0029					
	0,0002	0,0000	0,0020	0,0005	0,0008	0,0002
s^2	0,0036					
studentized residual	-0,55	0,12	1,68	-0,77	-1,04	1,06
	0,55	0,12	1,68	0,77	1,04	1,06
	1	1	0	1	1	1
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
	0,023	0,071		0,095	0,166	0,403
slope	2682,52457					
intercept	0,00060					
r^2	0,9527					

2. step outlier detection

mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00		0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,60	0,64		0,79	0,76	0,21
residuals	0,003	0,035		-0,021	-0,043	0,027
	0,0000	0,0004		0,0002	0,0006	0,0002
s^2	0,0014					
studentized residual	0,09	1,15		-0,64	-1,31	1,57
	0,09	1,15		0,64	1,31	1,57
	1	1	0	1	1	1
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
	0,023	0,071		0,095	0,166	0,403
slope	2682,52457	**3,73E-04**				
intercept	0,00060					
r^2	0,9527					

initial r2: 0,8386

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	0	1	1	1
albumin conc. [µM]	0,000	0,000		0,000	0,000	0,000
(1-fu)/fu	0,0	0,071		0,095	0,166	0,403
	2682,52457 w/o outlier		with outlier			
	0,02586	239,974	239,968	0,00 ok		
	0,9527 improvement?		no	3,4		
	accept outlier:		**no**			
abs(studentized residuals)	0,55	0,12		0,77	1,04	1,06
	7,4091E-06	1,33364E-05		4,32099E-05	7,77778E-05	0,00014
	0,0	0,1		0,1		0,4

checking effect of outlier removal on intercept estimation of 1/signal

	1	1	0	1	1	1
	0,000	0,000		0,000	0,000	0,000
	3,61E-03	3,78E-03		3,87E-03	4,12E-03	4,95E-03
	9,46885 w/o outlier		with outlier			
	0,00353	0,004	0,004	0,000 ok		
	0,9527 improvement		no			

second tier r2: 0,9527

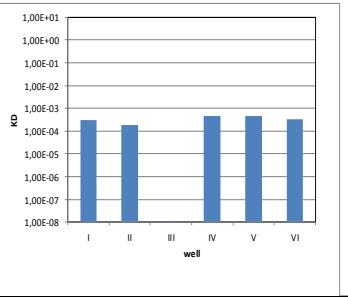
skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	0	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,887	4,668	4,236	4,566	4,287	3,563
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,113	0,332	0,764	0,434	0,713	1,437
KD	3,2E-04	1,8E-04		4,5E-04	4,6E-04	3,4E-04
	3,5E-04					
SE(KD)	5,095E-05					
fb	0,651	0,763		0,567	0,559	0,631
	63,4%					
SE(fb)	3,673%					
n	5					
	3,16E-04	1,83E-04	1,00E-12	4,50E-04	4,63E-04	3,44E-04



	test article No	2	SL 5				
	5,000 µM					0 nm / amu	
adj. Ref 1	1,500	M		av ref too low			
Ref 2	0,000	high ref diff	M	max ref ok			
Signal Reference:	1,500	1,500	1,500	1,500	1,500	1,500	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	1,100	na	na	na	na	na	ok

Ref 1 <> Ref 2

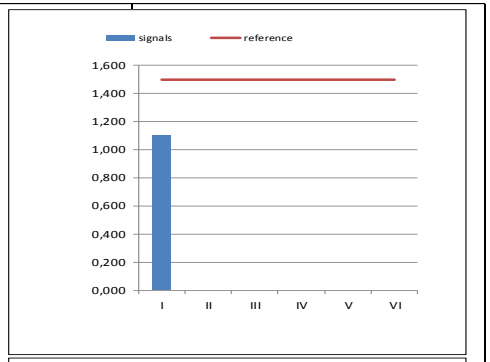
	missing values					
	1	0	0	0	0	0
	outlier detection					
consistency	ok	**inconsistent**	**inconsistent**	**inconsistent**	**inconsistent**	**inconsistent**
saturation	ok	**na**	**na**	**na**	**na**	**na**
statistics	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**#DIV/0!**	**na**

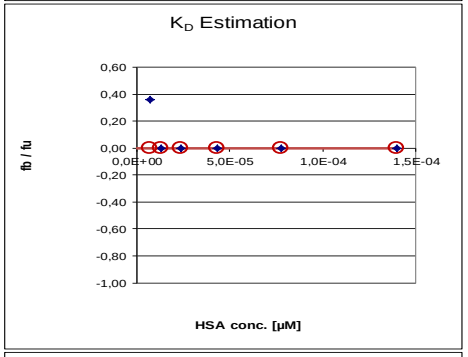
r^2= NaN

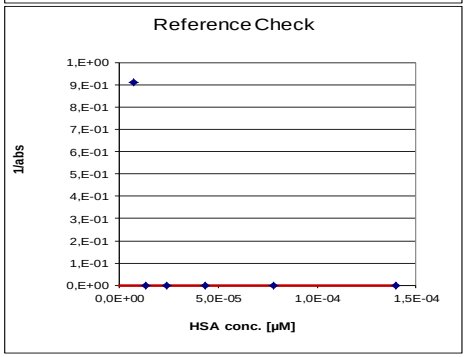
QC index: **0,0** **k_D= NaN** **fb=** **< 29,6%**

warning messages: #DIV/0!

		consistency check					
signal < reference	1	1	0	0	0	0	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	1	1	0	0	0	0	0
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		9,09E-01	#WERT!	#WERT!	#WERT!	#WERT!	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		26,7%	0,0%	0,0%	0,0%	0,0%	0,0%
(1-fu)/fu		0,364	0,000	0,000	0,000	0,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,364	0,000	0,000	0,000	0,000	0,000
slope		-1242,36766					
intercept		0,12391					
r^2		0,1785					
prediction: (1-fu)/fu		1,1E-01	1,1E-01	9,4E-02	7,0E-02	2,7E-02	-5,0E-02
outlier due to saturation and consistency		1	0	0	0	0	0
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06					
(1-fu)/fu		0,364					
slope		#DIV/0!					
intercept		#DIV/0!					
r^2		#DIV/0!					
prediction		#DIV/0!					
residuals							
standarized residuals							
Threshold		1,5					
statistical outlier	err	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0
		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
slope		#DIV/0!	**#DIV/0!**				
intercept		#DIV/0!					
r^2		#DIV/0!					
prediction	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
			outlier				
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0,000
			Binding QC				
no. of binding sites		1					
conc. protein-compound complex [µM]		1,33	0,00	0,00	0,00	0,00	0,00
free protein con. [µM]		6,08	13,34	24,01	43,21	77,78	140,00
free protein con. [M]	0	6,1E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
binding sites covered [%]		18,0%	0,0%	0,0%	0,0%	0,0%	0,0%
warning?							







reg	**#DIV/0!**	**NaN**
avg	**#DIV/0!**	0
choice	**NaN**	

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		9,09E-01	#WERT!	#WERT!	#WERT!	#WERT!	#WERT!
valid albumin conc. [µM]		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
valid 1/signal		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
slope		#DIV/0!					
intercept		#DIV/0!					
r^2		#DIV/0!					
predicted 1/signal	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Predicted Reference Value		#DIV/0!					
Actual Reference Value		1,50					
Relative deviation		-					

QC index: #DIV/0!

no. data points	#DIV/0!	reference	#WERT!	r^2 MA prediction	#DIV/0!	ref. Treatment	10	ref > signal	1	consistency	0	data points	#DIV/0!	#DIV/0!
		10,0%	#WERT!	0,9999	#DIV/0!	A		6		6		reference	#WERT!	#WERT!
6	#DIV/0!	20,0%	#WERT!	0,999	#DIV/0!	M	10	5		5		ref. Treatment		
5	#DIV/0!	50,0%	#WERT!	0,99	#DIV/0!	R		4		4		ref > signal	references e	X
4	#DIV/0!	100,0%	#WERT!	0,9	#DIV/0!	slopes	#DIV/0!	3		3		consistency	data not cor	X
3	#DIV/0!	200,0%	#WERT!	0,8	#DIV/0!	1/abs > 0	#DIV/0!	2		2		r^2 MA prediction	#DIV/0!	#DIV/0!
2	#DIV/0!	500,0%	#WERT!	0,7	#DIV/0!	c(lipid)/c(buffer) >0	#DIV/0!	1	1	intercept		slopes	#DIV/0!	#DIV/0!
1	#DIV/0!	<500%	#WERT!	0,6	#DIV/0!			0		#DIV/0!	#DIV/0!	intercept	#DIV/0!	#DIV/0!
0	#DIV/0!			0,5	#DIV/0!							binding		
				<0.5	#DIV/0!							summary	#DIV/0!	

warning messages

1. step outlier detection

mean x	0,000	n	1
$(x_i\text{-mean}(x))^2$	0,0E+00		
$S(x_i\text{-mean}(x))^2$	0,0E+00		
	#DIV/0!		
residuals			
	#DIV/0!		
	#WERT!		
s^2	#WERT!		
studentized residual	#WERT!		
	#WERT!		
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
slope	#DIV/0!					
intercept	#DIV/0!					
r^2	#DIV/0!					

2. step outlier detection

mean x	#DIV/0!	n	#DIV/0!
$(x_i\text{-mean}(x))^2$			
$S(x_i\text{-mean}(x))^2$	0,00		
residuals			
s^2	0,0000		
studentized residual			
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
slope	#DIV/0!	**#DIV/0!**				
intercept	#DIV/0!					
r^2	#DIV/0!					

initial r2: #DIV/0!

skip outlier detection if r 0,98

#DIV/0! outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

		#DIV/0!	0	0	0	0	0
albumin conc. [µM]		#DIV/0!					
(1-fu)/fu		#DIV/0!					
		#DIV/0!	w/o outlier	with outlier			
		#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
		#DIV/0!	improvemer	#DIV/0!	#DIV/0!		
			accept outli	**#WERT!**			
abs(studentized residuals)		#DIV/0!					#DIV/0!
		#DIV/0!					
		#DIV/0!					

second tier r2: #DIV/0!

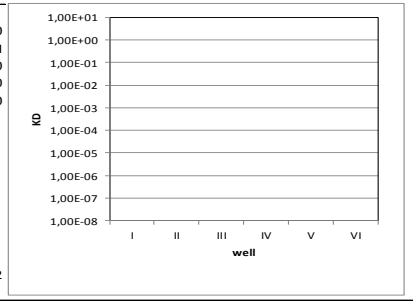
skip outlier detection if r 0,98

#DIV/0! outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	0
	I	II	III	IV	V	VI
[A] in [M]	3,667	5,000	5,000	5,000	5,000	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	1,333	0,000	0,000	0,000	0,000	0,000
KD	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
	#DIV/0!					
SE(KD)						
fb	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
	#DIV/0!					
SE(fb)						
n	0					
	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1,00E-12



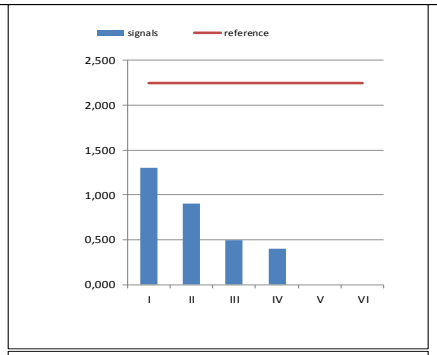
	test article No		3		**SL 4**			
		5,000 µM					0 nm / amu	
adj. Ref 1		2,400	A		av ref ok			
Ref 2		2,100	ref diff ok		max ref ok			
Signal Reference:		2,250	2,250	2,250	2,250	2,250	2,250	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		1,300	0,900	0,500	0,400 na	na		ok

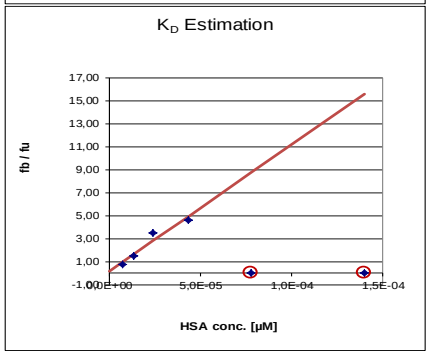
	missing values					
	1	1	1	1	0	0
	outlier detection					
consistency	ok	ok	ok	ok	**inconsistent**	**inconsistent**
saturation	ok	ok	ok	ok	**na**	**na**
statistics	ok	ok	ok	ok	**na**	**na**

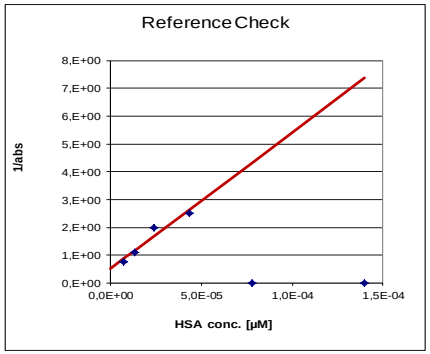
QC index: **7,4** **k_D= 9,10E-06** **fb=** **98,5% ± 0,093%** **r^2= 0,93265**

warning messages: references exceed signal: solubility problem? data not consistent,

			consistency check				
signal < reference	4	1	1	1	1	0	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	4	1	1	1	1	0	0
			removal of outliers due to saturation				
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		7,69E-01	1,11E+00	2,00E+00	2,50E+00	#WERT!	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		42,2%	60,0%	77,8%	82,2%	0,0%	0,0%
(1-fu)/fu		0,731	1,500	3,500	4,625	0,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,731	1,500	3,500	4,625	0,000	0,000
slope		-17135,32614					
intercept		2,59912					
r^2		0,2017					
prediction: (1-fu)/fu		2,5E+00	2,4E+00	2,2E+00	1,9E+00	1,3E+00	2,0E-01
outlier due to saturation and consistency		1	1	1	1	0	0
			statistical outlier detection & removal				
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
(1-fu)/fu		0,731	1,500	3,500	4,625		
slope		109941,50245					
intercept		0,17131					
r^2		0,9326					
prediction		0,986	1,638	2,811	4,922		
residuals		-0,255	-0,138	0,689	-0,297		
standarized residuals		0,000	0,000	1,484	0,000		
Threshold		1,5					
statistical outlier	4	1	1	1	1	0	0
		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
		0,731	1,500	3,500	4,625		
slope		109941,50245	**9,10E-06**				
intercept		0,17131					
r^2		0,9326					
prediction	0,171	0,986	1,638	2,811	4,922	8,722	15,563
			outlier				
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	0,000	0,000
			Binding QC				
no. of binding sites		1					
conc. protein-compound complex [µM]		2,11	3,00	3,89	4,11	0,00	0,00
free protein con. [µM]		5,30	10,34	20,12	39,10	77,78	140,00
free protein con. [M]	0	5,3E-06	1,0E-05	2,0E-05	3,9E-05	7,8E-05	1,4E-04
binding sites covered [%]		28,5%	22,5%	16,2%	9,5%	0,0%	0,0%
warning?							







reg	**9,10E-06**	**0,93265**	
avg	**7,09E-06**		1
choice	**9,10E-06**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		7,69E-01	1,11E+00	2,00E+00	2,50E+00	#WERT!	#WERT!
valid albumin conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
valid 1/signal		7,7E-01	1,1E+00	2,0E+00	2,5E+00		
slope		48862,88998					
intercept		0,52058					
r^2		0,9326					
predicted 1/signal	1	8,8E-01	1,2E+00	1,7E+00	2,6E+00	4,3E+00	7,4E+00
Predicted Reference Value		1,92					
Actual Reference Value		2,25					
Relative deviation		14,6%					

QC index: 7,4

no. data points	6	reference	9	r^2 MA prediction	7	ref. Treatment	10	ref > signal	6	consistency	3	warning messages		
		10,0%		0,9999		A	10	6		6		data points		
6		20,0%	9	0,999		M		5		5		reference		
5		50,0%	8	0,99		R		4	6	4	3	ref. Treatment		
4	6	100,0%	7	0,9	7	slopes	10	3		3		ref > signal references exceed	X	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency data not consisten	X	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction		
1		<500%		0,6	4			0		0,17	10	slopes		
0				0,5	3							intercept		
				<0,5								binding		

summary references exceed signal: solubility problem? data not cor

not consistent

1. step outlier detection

mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	2,1E-10	7,5E-11	4,1E-12	4,5E-10		
$S(x_i\text{-mean}(x))^2$	7,4E-10					
	0,46	0,65	0,74	0,14		
residuals	-0,255	-0,138	0,689	-0,297		
	0,2158					
	0,0325	0,0095	0,2377	0,0441		
s^2	0,3238					
studentized residual	-0,66	-0,30	1,40	-1,38		
	0,66	0,30	1,40	1,38		
	1	1	1	1	0	0
	7,4E-06	1,3E-05	2,4E-05	4,3E-05		
	0,731	1,500	3,500	4,625		
slope	109941,50245					
intercept	0,17131					
r^2	0,9326					

initial r2: 0,9326

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	0	0
albumin conc. [µM]	0,000	0,000	0,000	0,000		
(1-fu)/fu	0,7	1,500	3,500	4,625		
	109941,50245	w/o outlier	with outlier			
	0,17131	239,829	239,829	0,00 ok		
	0,9326	improvement?	no	1,0		
		accept outlier:	**na**			
abs(studentized residuals)	0,66	0,30	1,40	1,38		1,40
	7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05		
	0,7	1,5		4,6		

2. step outlier detection

mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00		
$S(x_i\text{-mean}(x))^2$	0,00					
	0,46	0,65	0,74	0,14		
residuals	-0,255	-0,138	0,689	-0,297		
	0,0325	0,0095	0,2377	0,0441		
s^2	0,3238					
studentized residual	-0,66	-0,30	1,40	-1,38		
	0,66	0,30	1,40	1,38		
	1	1	1	1	0	0
	7,4E-06	1,3E-05	2,4E-05	4,3E-05		
	0,731	1,500	3,500	4,625		
slope	109941,50245	**9,10E-06**				
intercept	0,17131					
r^2	0,9326					

second tier r2: 0,9326

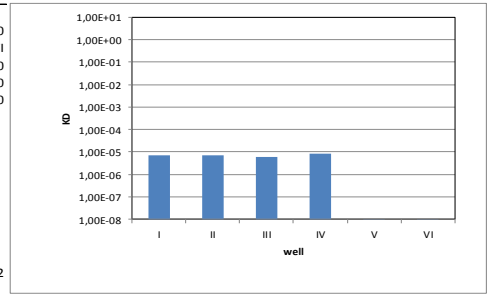
skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	1	1	0	0
	I	II	III	IV	V	VI
[A] in [M]	2,889	2,000	1,111	0,889	5,000	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,111	3,000	3,889	4,111	0,000	0,000
KD	7,2E-06	6,9E-06	5,7E-06	8,5E-06		
	7,1E-06					
SE(KD)	5,573E-07					
fb	0,988	0,988	0,990	0,986		
	98,8%					
SE(fb)	0,093%					
n	4					
	7,25E-06	6,89E-06	5,75E-06	8,45E-06	1,00E-12	1,00E-12



test article No 4 **SL 6**

		5,000 µM				0 nm / amu	
adj. Ref 1		69,600	A		av ref ok		
Ref 2		67,800	ref diff ok		max ref ok		
Signal Reference:		68,700	68,700	68,700	68,700	68,700	68,700
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		60,100	60,600	43,200	42,400	24,000	16,500	ok

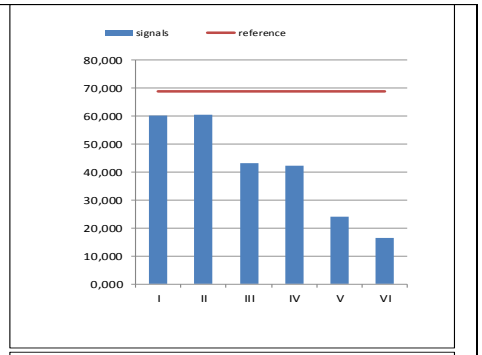
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

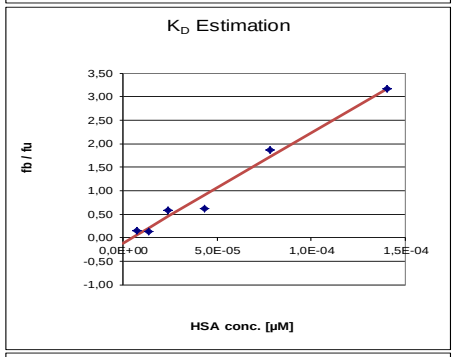
r^2 = **0,98159**

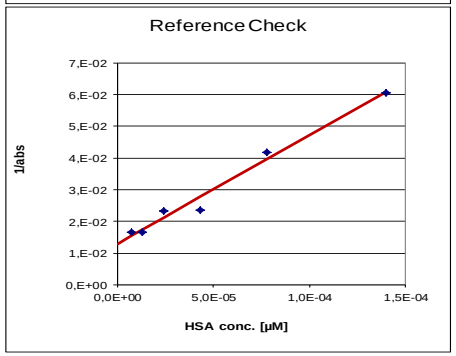
QC index: **9,4** **k_D= 4,25E-05** **fb=** **93,3% ± 1,255%**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,66E-02	1,65E-02	2,31E-02	2,36E-02	4,17E-02	6,06E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		12,5%	11,8%	37,1%	38,3%	65,1%	76,0%
(1-fu)/fu		0,143	0,134	0,590	0,620	1,863	3,164
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,143	0,134	0,590	0,620	1,863	3,164
slope		23511,64461					
intercept		-0,11249					
r^2		0,9816					
prediction: (1-fu)/fu		6,2E-02	2,0E-01	4,5E-01	9,0E-01	1,7E+00	3,2E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,143	0,134	0,590	0,620	1,863	3,164
slope		23511,64461					
intercept		-0,11249					
r^2		0,9816					
prediction		0,062	0,201	0,452	0,903	1,716	3,179
residuals		0,081	-0,067	0,138	-0,283	0,146	-0,016
standarized residuals		0,501	0,000	0,851	0,000	0,900	0,000
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
		0,143	0,134	0,590	0,620	1,863	3,164
slope		23511,64461	**4,25E-05**				
intercept		-0,11249					
r^2		0,9816					
prediction	-0,112	0,062	0,201	0,452	0,903	1,716	3,179
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,63	0,59	1,86	1,91	3,25	3,80
free protein con. [µM]		6,78	12,75	22,15	41,30	74,52	136,20
free protein con. [M]	0	6,8E-06	1,3E-05	2,2E-05	4,1E-05	7,5E-05	1,4E-04
binding sites covered [%]		8,4%	4,4%	7,7%	4,4%	4,2%	2,7%
warning?							







reg	**4,25E-05**	**0,98159**	
avg	**5,50E-05**		1
choice	**4,25E-05**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,66E-02	1,65E-02	2,31E-02	2,36E-02	4,17E-02	6,06E-02
valid albumin conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal		1,7E-02	1,7E-02	2,3E-02	2,4E-02	4,2E-02	6,1E-02
slope		342,23646					
intercept		0,01292					
r^2		0,9816					
predicted 1/signal	0	1,5E-02	1,7E-02	2,1E-02	2,8E-02	4,0E-02	6,1E-02
Predicted Reference Value		77,41					
Actual Reference Value		68,70					
Relative deviation		11,2%					

QC index: 9,4

no. data points	10	reference	9	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%		0,9999		A	10	6	10	6	10	data points	
6	10	20,0%	9	0,999		M		5		5		reference	
5		50,0%	8	0,99		R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		-0,11	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,081	-0,067	0,138	-0,283	0,146	-0,016
	0,0264					
	0,0017	0,0011	0,0048	0,0200	0,0054	0,0001
s^2	0,0330					
studentized residual	0,54	-0,44	0,86	-1,71	0,91	-0,19
	0,54	0,44	0,86	1,71	0,91	0,19
	1	1	1	1	1	1
	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
	0,143	0,134	0,590	0,620	1,863	3,164
slope	23511,64461					
intercept	-0,11249					
r^2	0,9816					
	2. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,081	-0,067	0,138	-0,283	0,146	-0,016
	0,0017	0,0011	0,0048	0,0200	0,0054	0,0001
s^2	0,0330					
studentized residual	0,54	-0,44	0,86	-1,71	0,91	-0,19
	0,54	0,44	0,86	1,71	0,91	0,19
	1	1	1	1	1	1
	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
	0,143	0,134	0,590	0,620	1,863	3,164
slope	23511,64461	**4,25E-05**				
intercept	-0,11249					
r^2	0,9816					

initial r2:	0,9816	
skip outlier detection if r2 >	0,98	
	omit	outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

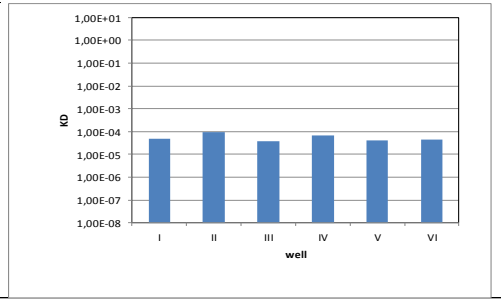
checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1
albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000
(1-fu)/fu	0,1	0,134	0,590	0,620	1,863	3,164
	23511,64461	w/o outlier	with outlier			
	-0,12638	240,126	240,112	0,00 ok		
	0,9816	improvement?	no	1,0		
		accept outlier:	**na**			
abs(studentized residuals)	0,54	0,44	0,86	1,71	0,91	0,19	1,71
	7,4091E-06	1,33364E-05	2,40055E-05		7,77778E-05	0,00014	
	0,1	0,1	0,6		1,9	3,2	

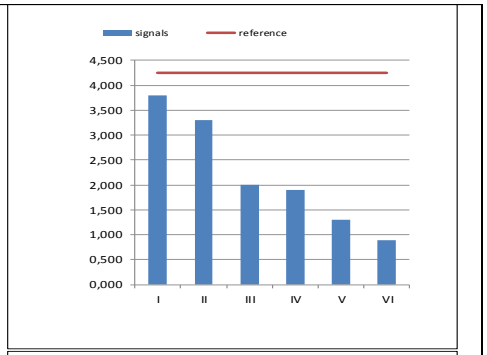
second tier r2:	0,9816	
skip outlier detection if r2 >	0,98	
	omit	outlier detection

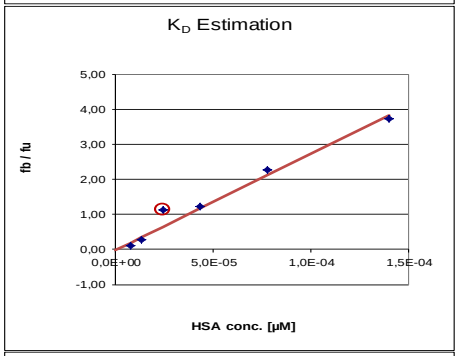
no outliers will be recorded if there are only 3 or less points left at step 1 or 2

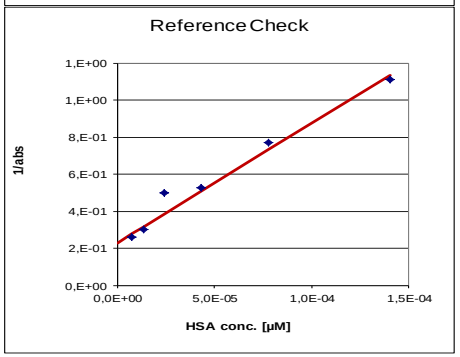
	Standard Error					
	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,374	4,410	3,144	3,086	1,747	1,201
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,626	0,590	1,856	1,914	3,253	3,799
KD	4,7E-05	9,5E-05	3,8E-05	6,7E-05	4,0E-05	4,3E-05
	5,5E-05					
SE(KD)	9,117E-06					
fb	0,925	0,860	0,940	0,898	0,936	0,932
	91,5%					
SE(fb)	1,255%					
n	6					
	4,74E-05	9,54E-05	3,75E-05	6,66E-05	4,00E-05	4,31E-05



	test article No	5		**SL 7**			
		5,000 µM				0 nm / amu	
adj. Ref 1		4,700	A		av ref ok		
Ref 2		3,800	ref diff ok		max ref ok		
Signal Reference:		4,250	4,250	4,250	4,250	4,250	4,250
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		3,800	3,300	2,000	1,900	1,300	0,900 ok
				missing values			
		1	1	1	1	1	1
				outlier detection			
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		ok	ok	**outlier**	ok	ok	ok
							r^2= **0,99481**
QC index:	**9,6**	**k_D= 3,65E-05**		**fb=**	**94,2% ± 0,695%**		
warning messages:							
			consistency check				
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
			removal of outliers due to saturation				
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,63E-01	3,03E-01	5,00E-01	5,26E-01	7,69E-01	1,11E+00
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		10,6%	22,4%	52,9%	55,3%	69,4%	78,8%
(1-fu)/fu		0,118	0,288	1,125	1,237	2,269	3,722
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,118	0,288	1,125	1,237	2,269	3,722
slope		26393,53144					
intercept		0,11501					
r^2		0,9749					
prediction: (1-fu)/fu		3,1E-01	4,7E-01	7,5E-01	1,3E+00	2,2E+00	3,8E+00
outlier due to saturation and consistency		1	1	1	1	1	1
			statistical outlier detection & removal				
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,118	0,288	1,125	1,237	2,269	3,722
slope		26393,53144					
intercept		0,11501					
r^2		0,9749					
prediction		0,311	0,467	0,749	1,255	2,168	3,810
residuals		-0,192	-0,179	0,376	-0,019	0,101	-0,088
standarized residuals		0,000	0,000	1,759	0,000	0,474	0,000
Threshold		1,5					
statistical outlier	5	1	1	0	1	1	1
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
		0,118	0,288		1,237	2,269	3,722
slope		27418,68036	**3,65E-05**				
intercept		-0,01803					
r^2		0,9948					
prediction	-0,018	0,185	0,348	0,640	1,167	2,115	3,821
			outlier				
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	1,125	-5,000	-5,000	-5,000
			Binding QC				
no. of binding sites		1					
conc. protein-compound complex [µM]		0,53	1,12	2,65	2,76	3,47	3,94
free protein con. [µM]		6,88	12,22	21,36	40,45	74,31	136,06
free protein con. [M]	0	6,9E-06	1,2E-05	2,1E-05	4,0E-05	7,4E-05	1,4E-04
binding sites covered [%]		7,1%	8,4%	11,0%	6,4%	4,5%	2,8%
warning?							







reg	**3,65E-05**	**0,99481**	
avg	**4,05E-05**		1
choice	**3,65E-05**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,63E-01	3,03E-01	5,00E-01	5,26E-01	7,69E-01	1,11E+00
valid albumin conc. [µM]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
valid 1/signal		2,6E-01	3,0E-01		5,3E-01	7,7E-01	1,1E+00
slope		6451,45420					
intercept		0,23105					
r^2		0,9948					
predicted 1/signal	0	2,8E-01	3,2E-01	3,9E-01	5,1E-01	7,3E-01	1,1E+00
Predicted Reference Value		4,33					
Actual Reference Value		4,25					
Relative deviation		1,8%					

QC index: 9,6

no. data points	9	reference	10	r^2 MA prediction	8	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%	10	0,9999		A	10	6	10	6	10	data points	
6		20,0%	9	0,999		M		5		5		reference	
5	9	50,0%	8	0,99	8	R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		-0,02	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	-0,192	-0,179	0,376	-0,019	0,101	-0,088
	0,0458					
	0,0092	0,0080	0,0354	0,0001	0,0026	0,0019
s^2	0,0573					
studentized residual	-0,97	-0,88	1,79	-0,09	0,48	-0,80
	0,97	0,88	1,79	0,09	0,48	0,80
	1	1	0	1	1	1
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
	0,118	0,288		1,237	2,269	3,722
slope	27418,68036					
intercept	-0,01803					
r^2	0,9948					

initial r2: 0,9749

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	0	1	1	1
albumin conc. [µM]	0,000	0,000		0,000	0,000	0,000
(1-fu)/fu	0,1	0,288		1,237	2,269	3,722
	27418,68036 w/o outlier		with outlier			
	-0,10998	240,110	239,885	0,00 ok		
	0,9948 improvement?		no	4,8		
	accept outlier:		**no**			
abs(studentized residuals)	0,97	0,88		0,09	0,48	0,80	0,97
	7,4091E-06	1,33364E-05		4,32099E-05	7,77778E-05	0,00014	
		0,3		1,2	2,3	3,7	

2. step outlier detection

mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00		0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,60	0,64		0,79	0,76	0,21
residuals	-0,067	-0,060		0,070	0,155	-0,098
	0,0015	0,0012		0,0016	0,0080	0,0032
s^2	0,0155					
studentized residual	-0,69	-0,60		0,64	1,42	-1,72
	0,69	0,60		0,64	1,42	1,72
	1	1	0	1	1	1
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
	0,118	0,288		1,237	2,269	3,722
slope	27418,68036	**3,65E-05**				
intercept	-0,01803					
r^2	0,9948					

second tier r2: 0,9948

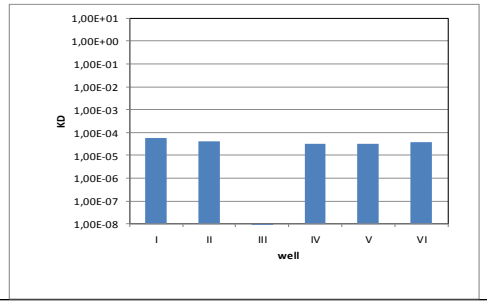
skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	0	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,471	3,882	2,353	2,235	1,529	1,059
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,529	1,118	2,647	2,765	3,471	3,941
KD	5,8E-05	4,2E-05		3,3E-05	3,3E-05	3,7E-05
	4,1E-05					
SE(KD)	4,743E-06					
fb	0,910	0,933		0,947	0,947	0,941
	93,6%					
SE(fb)	0,695%					
n	5					
	5,81E-05	4,24E-05	1,00E-12	3,27E-05	3,27E-05	3,66E-05



		test article No	6		SL 14			
		5,000 µM					0 nm / amu	
adj. Ref 1		90,700	R		av ref too low			
Ref 2		83,300	ref diff ok		max ref too low	R		
Signal Reference:		95,500	95,500	95,500	95,500	95,500	95,500	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		95,500	71,700 na		45,000	35,800	18,300	ok

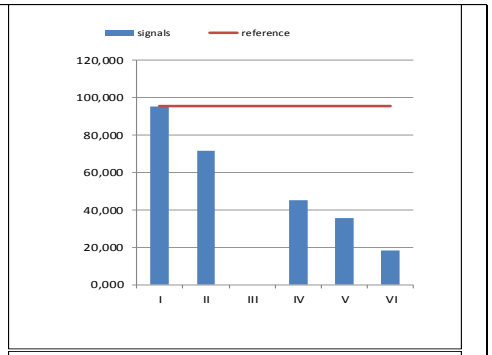
	missing values					
	1	1	0	1	1	1
	outlier detection					
consistency	ok	ok	**inconsistent**	ok	ok	ok
saturation	**reference**	ok	**na**	ok	ok	ok
statistics	ok	ok	**na**	ok	**outlier**	ok

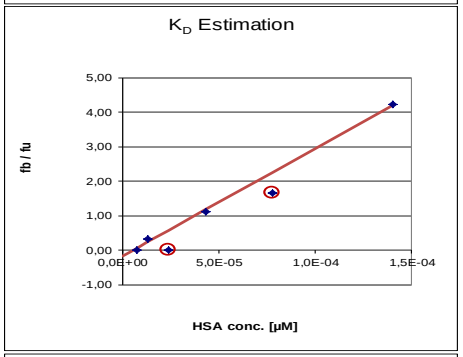
r^2= **0,99871**

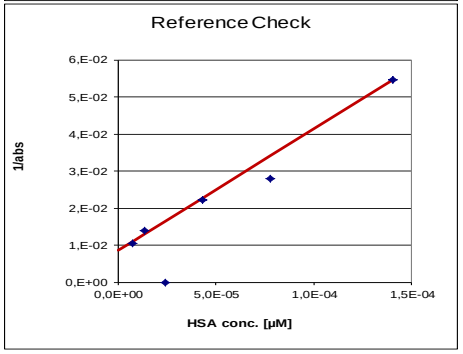
QC index: **8,1** **k_D= 3,19E-05** **fb=** **94,9% ± 0,206%**

warning messages: references exceed signal: solubility problem? data not consistent,

		consistency check					
signal < reference	4	0	1	0	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	0	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,05E-02	1,39E-02	#WERT!	2,22E-02	2,79E-02	5,46E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	24,9%	0,0%	52,9%	62,5%	80,8%
(1-fu)/fu		0,000	0,332	0,000	1,122	1,668	4,219
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,332	0,000	1,122	1,668	4,219
slope		31317,99469					
intercept		-0,37246					
r^2		0,9641					
prediction: (1-fu)/fu		-1,4E-01	4,5E-02	3,8E-01	9,8E-01	2,1E+00	4,0E+00
outlier due to saturation and consistency		1	1	0	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,000	0,332		1,122	1,668	4,219
slope		30284,83395					
intercept		-0,23838					
r^2		0,9750					
prediction		-0,014	0,166		1,070	2,117	4,001
residuals		0,014	0,166		0,052	-0,450	0,217
standarized residuals		0,053	0,629		0,197	0,000	0,821
Threshold		1,5					
statistical outlier	4	1	1	0	1	0	1
		7,4E-06	1,3E-05		4,3E-05		1,4E-04
		0,000	0,332		1,122		4,219
slope		31350,44746	**3,19E-05**				
intercept		-0,18034					
r^2		0,9987					
prediction	-0,180	0,052	0,238	0,572	1,174	2,258	4,209
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	0,000	-5,000	1,668	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	1,25	0,00	2,64	3,13	4,04
free protein con. [µM]		7,41	12,09	24,01	40,57	74,65	135,96
free protein con. [M]	0	7,4E-06	1,2E-05	2,4E-05	4,1E-05	7,5E-05	1,4E-04
binding sites covered [%]		0,0%	9,3%	0,0%	6,1%	4,0%	2,9%
warning?							







reg	**3,19E-05**	**0,99871**	
avg	**3,49E-05**		1
choice	**3,19E-05**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,05E-02	1,39E-02	#WERT!	2,22E-02	2,79E-02	5,46E-02
valid albumin conc. [µM]		7,4E-06	1,3E-05		4,3E-05		1,4E-04
valid 1/signal		1,0E-02	1,4E-02		2,2E-02		5,5E-02
slope		328,27694					
intercept		0,00858					
r^2		0,9987					
predicted 1/signal	0	1,1E-02	1,3E-02	1,6E-02	2,3E-02	3,4E-02	5,5E-02
Predicted Reference Value		116,51					
Actual Reference Value		95,50					
Relative deviation		18,0%					

QC index: 8,1

no. data points	6	reference	9	r^2 MA prediction	8	ref. Treatment	10	ref > signal	6	consistency	6		
		10,0%		0,9999		A		6		6			
6		20,0%	9	0,999		M		5		5	6		
5		50,0%	8	0,99	8	R	10	4	6	4			
4	6	100,0%	7	0,9	7	slopes	10	3		3			
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept			
1		<500%		0,6	4			0		-0,18	10		
0				0,5	3								
				<0.5									

warning messages

data points	
reference	
ref. Treatment	
ref > signal references exceed	X
consistency data not consisten	X
r^2 MA prediction	
slopes	
intercept	
binding	

summary references exceed signal: solubility problem? data not cor

data not consistent

1. step outlier detection

mean x		0,000		n	5	
$(x_i\text{-mean}(x))^2$		2,4E-09	1,8E-09		1,7E-10	4,6E-10	7,0E-09
$S(x_i\text{-mean}(x))^2$		1,2E-08					
		0,60	0,64		0,79	0,76	0,21
residuals		0,014	0,166		0,052	-0,450	0,217
		0,0699					
		0,0001	0,0092		0,0009	0,0674	0,0157
s^2		0,0933					
studentized residual		0,06	0,68		0,19	-1,69	1,55
		0,06	0,68		0,19	1,69	1,55
		1	1	0	1	0	1
		7,4E-06	1,3E-05		4,3E-05		1,4E-04
		0,000	0,332		1,122		4,219
slope		31350,44746					
intercept		-0,18034					
r^2		0,9987					

initial r2: 0,9750

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	0	1	0	1
albumin conc. [µM]	0,000	0,000		0,000		0,000
(1-fu)/fu	0,0	0,332		1,122		4,219
	31350,44746	w/o outlier		with outlier		
	-0,14977	240,150	240,238	0,00	ok	
	0,9987	improvement?	yes	19,4		
		accept outlier:	**yes**			
abs(studentized residuals)	0,06	0,68		0,19		1,55	0,68
	7,4091E-06	1,33364E-05		4,32099E-05		0,00014	
	0,0			1,1		4,2	

2. step outlier detection

mean x		0,000		n	4	
$(x_i\text{-mean}(x))^2$		0,00	0,00		0,00		0,00
$S(x_i\text{-mean}(x))^2$		0,00					
		0,58	0,62		0,74		0,05
residuals		-0,052	0,094		-0,052		0,010
		0,0013	0,0044		0,0014		0,0000
s^2		0,0072					
studentized residual		-0,80	1,41		-0,71		0,53
		0,80	1,41		0,71		0,53
		1	1	0	1	0	1
		7,4E-06	1,3E-05		4,3E-05		1,4E-04
		0,000	0,332		1,122		4,219
slope		31350,44746	**3,19E-05**				
intercept		-0,18034					
r^2		0,9987					

second tier r2: 0,9987

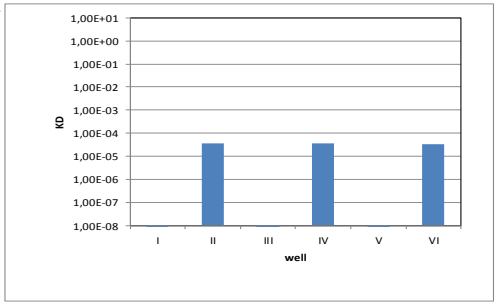
skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	0	1	0	1	0	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	3,754	5,000	2,356	1,874	0,958
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	1,246	0,000	2,644	3,126	4,042
KD		3,6E-05		3,6E-05		3,2E-05
	3,5E-05					
SE(KD)	1,355E-06					
fb		0,942		0,942		0,948
	94,4%					
SE(fb)	0,206%					
n	3					
	1,00E-12	3,64E-05	1,00E-12	3,61E-05	1,00E-12	3,22E-05



test article No 7 **SL 1**

5,000 µM 0 nm / amu

adj. Ref 1	19,500	R		av ref too low			
Ref 2	8,800	high ref diff	M	max ref too low	R		
Signal Reference:	31,000	31,000	31,000	31,000	31,000	31,000	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	31,000	30,500	17,000	16,400	12,600	18,000	ok

Ref 1 <> Ref 2

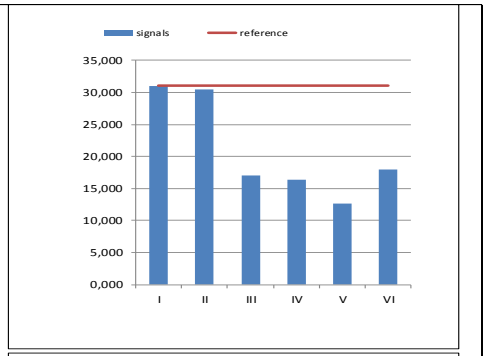
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	**inconsistent**
saturation	**reference**	ok	ok	ok	ok	**na**
statistics	ok	ok	**outlier**	ok	ok	**na**

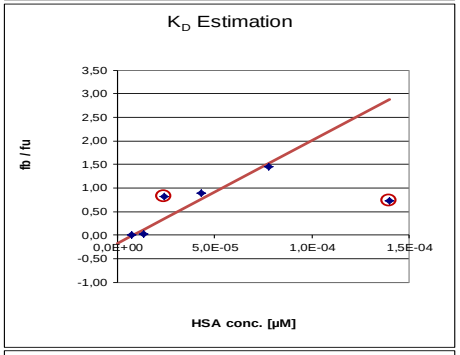
r^2= 0,98116

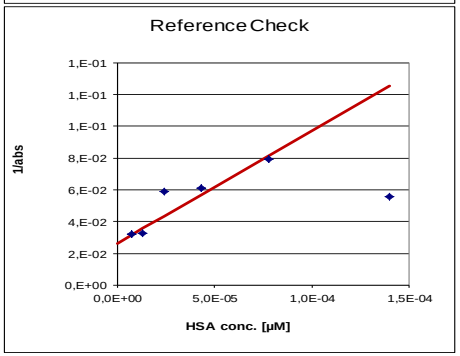
QC index: 8,1 **k_D= 4,57E-05** **fb= 92,8% ± 16,758%**

warning messages: data not consistent,

		consistency check					
signal < reference	5	0	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	1	1	0
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,23E-02	3,28E-02	5,88E-02	6,10E-02	7,94E-02	5,56E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	1,6%	45,2%	47,1%	59,4%	41,9%
(1-fu)/fu		0,000	0,016	0,824	0,890	1,460	0,722
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,016	0,824	0,890	1,460	0,722
slope		5787,55190					
intercept		0,35720					
r^2		0,2713					
prediction: (1-fu)/fu		4,0E-01	4,3E-01	5,0E-01	6,1E-01	8,1E-01	1,2E+00
outlier due to saturation and consistency		1	1	1	1	1	0
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
(1-fu)/fu		0,000	0,016	0,824	0,890	1,460	
slope		20509,16477					
intercept		-0,04174					
r^2		0,8671					
prediction		0,110	0,232	0,451	0,844	1,553	
residuals		-0,110	-0,215	0,373	0,046	-0,093	
standarized residuals		0,000	0,000	1,634	0,201	0,000	
Threshold		1,5					
statistical outlier	4	1	1	0	1	1	0
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	
		0,000	0,016		0,890	1,460	
slope		21871,99707	**4,57E-05**				
intercept		-0,18326					
r^2		0,9812					
prediction	-0,183	-0,021	0,108	0,342	0,762	1,518	2,879
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	0,824	-5,000	-5,000	0,722
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,08	2,26	2,35	2,97	2,10
free protein con. [µM]		7,41	13,26	21,75	40,86	74,81	137,90
free protein con. [M]	0	7,4E-06	1,3E-05	2,2E-05	4,1E-05	7,5E-05	1,4E-04
binding sites covered [%]		0,0%	0,6%	9,4%	5,4%	3,8%	1,5%
warning?							







reg	**4,57E-05**	**0,98116**	
avg	**3,02E-04**		1
choice	**4,57E-05**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,23E-02	3,28E-02	5,88E-02	6,10E-02	7,94E-02	5,56E-02
valid albumin conc. [µM]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	
valid 1/signal		3,2E-02	3,3E-02		6,1E-02	7,9E-02	
slope		705,54829					
intercept		0,02635					
r^2		0,9812					
predicted 1/signal	0	3,2E-02	3,6E-02	4,3E-02	5,7E-02	8,1E-02	1,3E-01
Predicted Reference Value		37,96					
Actual Reference Value		31,00					
Relative deviation		18,3%					

QC index: 8,1

no. data points	6	reference	9	r^2 MA prediction	7	ref. Treatment	10	ref > signal	8	consistency	6	warning messages						
		10,0%		0,9999		A		6		6		data points						
6		20,0%	9	0,999		M		5	8	5	6	reference						
5		50,0%	8	0,99		R	10	4		4		ref. Treatment						
4	6	100,0%	7	0,9	7	slopes	10	3		3		ref > signal						
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	data not consisten				X	
2		500,0%	3	0,7	5	pid)/c(buffer) >0	10	1		intercept		r^2 MA prediction						
1		<500%		0,6	4			0		-0,18	10	slopes						
0				0,5	3							intercept						
				<0.5								binding						
												summary	data not consistent,					

	1. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	6,6E-10	3,9E-10	8,4E-11	1,0E-10	2,0E-09	
$S(x_i\text{-mean}(x))^2$	3,2E-09					
	0,60	0,68	0,77	0,77	0,18	
residuals	-0,110	-0,215	0,373	0,046	-0,093	
	0,0521					
	0,0040	0,0155	0,0464	0,0007	0,0029	
s^2	0,0695					
studentized residual	-0,54	-0,99	1,61	0,20	-0,82	
	0,54	0,99	1,61	0,20	0,82	
	1	1	0	1	1	0
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	
	0,000	0,016		0,890	1,460	
slope	21871,99707					
intercept	-0,18326					
r^2	0,9812					
	2. step outlier detection					
mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	0,00	0,00		0,00	0,00	
$S(x_i\text{-mean}(x))^2$	0,00					
	0,50	0,59		0,73	0,18	
residuals	0,021	-0,092		0,128	-0,058	
	0,0002	0,0042		0,0082	0,0017	
s^2	0,0144					
studentized residual	0,25	-1,00		1,25	-1,14	
	0,25	1,00		1,25	1,14	
	1	1	0	1	1	0
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	
	0,000	0,016		0,890	1,460	
slope	21871,99707	**4,57E-05**				
intercept	-0,18326					
r^2	0,9812					

initial r2: 0,8671

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	0	1	1	0
albumin conc. [µM]	0,000	0,000		0,000	0,000	
(1-fu)/fu	0,0	0,016		0,890	1,460	
	21871,99707 w/o outlier		with outlier			
	-0,18326	240,183	240,042	0,00 ok		
	0,9812 improvement?		no	7,1		
	accept outlier:		**no**			
abs(studentized residuals)	0,54	0,99		0,20	0,82	0,99
	7,4091E-06	1,33364E-05		4,32099E-05	7,77778E-05	
	0,0			0,9	1,5	

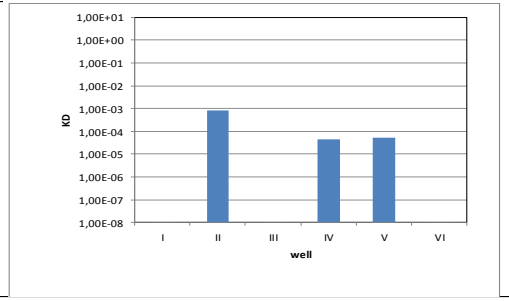
second tier r2: 0,9812

skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	0	1	0	1	1	0
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,919	2,742	2,645	2,032	2,903
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	0,081	2,258	2,355	2,968	2,097
KD		8,1E-04		4,6E-05	5,1E-05	
	3,0E-04					
SE(KD)	2,534E-04					
fb		0,421		0,928	0,920	
	75,6%					
SE(fb)	16,758%					
n	3					
	1,00E-12	8,09E-04	1,00E-12	4,59E-05	5,12E-05	1,00E-12



	test article No		8		**SL 8**		
		5,000 µM				0 nm / amu	
adj. Ref 1		23,000	R		av ref too low		
Ref 2		34,900	high ref diff	M	max ref too low	R	
Signal Reference:		38,700	38,700	38,700	38,700	38,700	38,700
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		38,700	16,900	13,200	12,700	9,200	6,000 ok

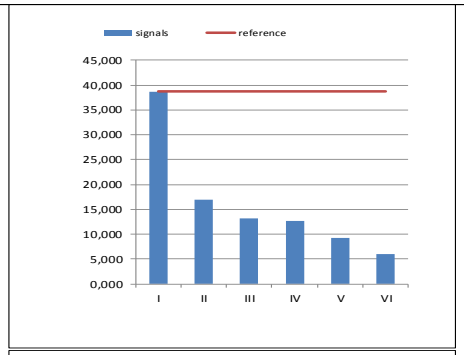
Ref 1 <> Ref 2

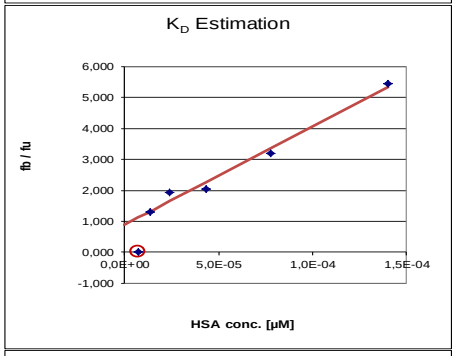
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	**reference**	ok	ok	ok	ok	ok
statistics	**outlier**	ok	ok	ok	ok	ok

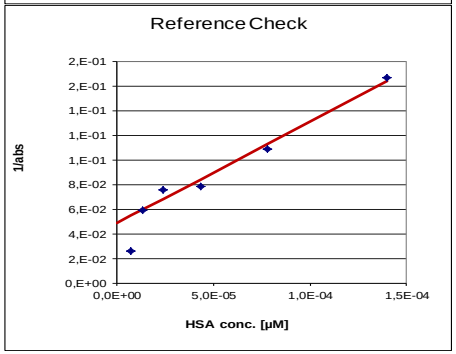
QC index: **8,2** **k_D= 3,14E-05** **fb=** **94,9% ± 0,537%** **r^2= 0,98505**

warning messages:

		consistency check					
signal < reference	5	0	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,58E-02	5,92E-02	7,58E-02	7,87E-02	1,09E-01	1,67E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	56,3%	65,9%	67,2%	76,2%	84,5%
(1-fu)/fu		0,000	1,290	1,932	2,047	3,207	5,450
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	1,290	1,932	2,047	3,207	5,450
slope		35684,48648					
intercept		0,50257					
r^2		0,9408					
prediction: (1-fu)/fu		7,7E-01	9,8E-01	1,4E+00	2,0E+00	3,3E+00	5,5E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,000	1,290	1,932	2,047	3,207	5,450
slope		35684,48648					
intercept		0,50257					
r^2		0,9408					
prediction		0,767	0,978	1,359	2,044	3,278	5,498
residuals		-0,767	0,311	0,573	0,003	-0,072	-0,048
standarized residuals		0,000	0,689	1,267	0,006	0,000	0,000
Threshold		1,5					
statistical outlier	5	0	1	1	1	1	1
			1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
			1,290	1,932	2,047	3,207	5,450
slope		31856,71300	**3,14E-05**				
intercept		0,88435					
r^2		0,9850					
prediction	0,884	1,120	1,309	1,649	2,261	3,362	5,344
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	2,82	3,29	3,36	3,81	4,22
free protein con. [µM]		7,41	10,52	20,71	39,85	73,97	135,78
free protein con. [M]	0	7,4E-06	1,1E-05	2,1E-05	4,0E-05	7,4E-05	1,4E-04
binding sites covered [%]		0,0%	21,1%	13,7%	7,8%	4,9%	3,0%
warning?							







reg	**3,14E-05**	**0,98505**	
avg	**1,73E-05**		1
choice	**3,14E-05**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,58E-02	5,92E-02	7,58E-02	7,87E-02	1,09E-01	1,67E-01
valid albumin conc. [µM]			1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal			5,9E-02	7,6E-02	7,9E-02	1,1E-01	1,7E-01
slope		823,17088					
intercept		0,04869					
r^2		0,9850					
predicted 1/signal	0	5,5E-02	6,0E-02	6,8E-02	8,4E-02	1,1E-01	1,6E-01
Predicted Reference Value		20,54					
Actual Reference Value		38,70					
Relative deviation		46,9%					

QC index: 8,2

no. data points	9	reference	8	r^2 MA prediction	7	ref. Treatment	10	ref > signal	8	consistency	10	warning messages
		10,0%		0,9999		A		6		6	10	data points
6		20,0%		0,999		M		5	8	5		reference
5	9	50,0%	8	0,99		R	10	4		4		ref. Treatment
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency
2		500,0%	3	0,7	5	:(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction
1		<500%		0,6	4			0		0,88	5	slopes
0				0,5	3							intercept
				<0.5								binding
												summary

	1. step outlier detection					
mean x	0,000	n	6			
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	-0,767	0,311	0,573	0,003	-0,072	-0,048
	0,2041					
	0,1471	0,0243	0,0820	0,0000	0,0013	0,0006
s^2	0,2552					
studentized residual	-1,84	0,73	1,29	0,01	-0,16	-0,21
	1,84	0,73	1,29	0,01	0,16	0,21
	0	1	1	1	1	1
		1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
		1,290	1,932	2,047	3,207	5,450
slope	31856,71300					
intercept	0,88435					
r^2	0,9850					
	2. step outlier detection					
mean x	0,000	n	5			
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
		0,60	0,68	0,77	0,77	0,18
residuals		-0,019	0,283	-0,214	-0,156	0,106
		0,0001	0,0266	0,0152	0,0081	0,0037
s^2	0,0538					
studentized residual		-0,11	1,48	-1,05	-0,77	1,06
		0,11	1,48	1,05	0,77	1,06
	0	1	1	1	1	1
		1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
		1,290	1,932	2,047	3,207	5,450
slope	31856,71300	**3,14E-05**				
intercept	0,88435					
r^2	0,9850					

initial r2: 0,9408

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	0	1	1	1	1	1
albumin conc. [µM]		0,000	0,000	0,000	0,000	0,000
(1-fu)/fu		1,290	1,932	2,047	3,207	5,450
	31856,71300 w/o outlier		with outlier			
	1,03274	238,967	239,497	0,00 ok		
	0,9850 improvement?		yes	4,0		
	accept outlier:		**yes**			
abs(studentized residuals)		0,73	1,29	0,01	0,16	0,21	1,29
		1,33364E-05	2,40055E-05	4,32099E-05	7,77778E-05	0,00014	
		1,3		2,0	3,2	5,5	

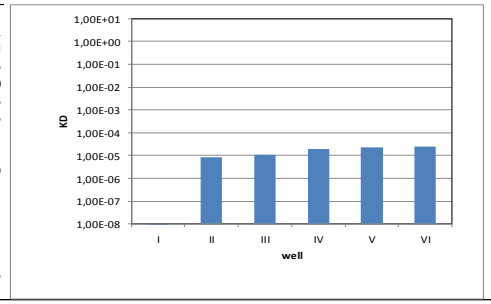
second tier r2: 0,9850

skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	0	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	2,183	1,705	1,641	1,189	0,775
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	2,817	3,295	3,359	3,811	4,225
KD		8,2E-06	1,1E-05	1,9E-05	2,3E-05	2,5E-05
	1,7E-05					
SE(KD)	3,338E-06					
fb		0,986	0,982	0,968	0,962	0,959
	97,2%					
SE(fb)	0,537%					
n	5					
	1,00E-12	8,16E-06	1,07E-05	1,95E-05	2,31E-05	2,49E-05



test article No 9 **SL 12**

		5,000 µM				0 nm / amu	
adj. Ref 1		3,500	R		av ref too low		
Ref 2		4,100	ref diff ok		max ref too low R		
Signal Reference:		5,000	5,000	5,000	5,000	5,000	5,000
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		5,000	4,600	2,200	1,100	0,600 na	ok

-

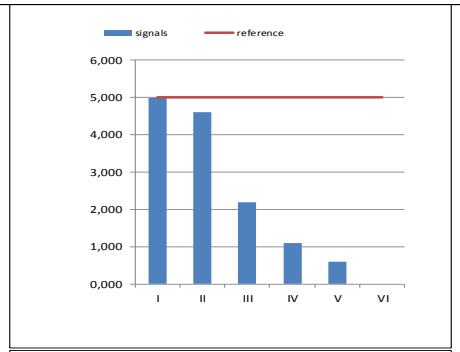
		missing values					
		1	1	1	1	1	0
		outlier detection					
consistency		ok	ok	ok	ok	ok	**inconsistent**
saturation		**reference**	ok	ok	ok	ok	**na**
statistics		ok	ok	ok	ok	ok	**na**

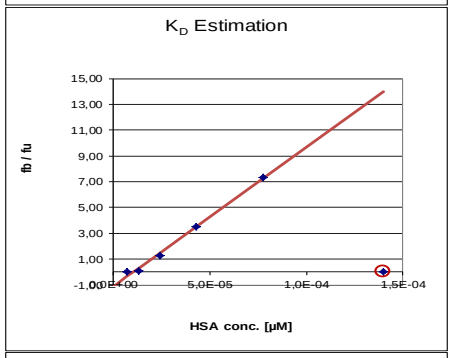
r^2= **0,99477**

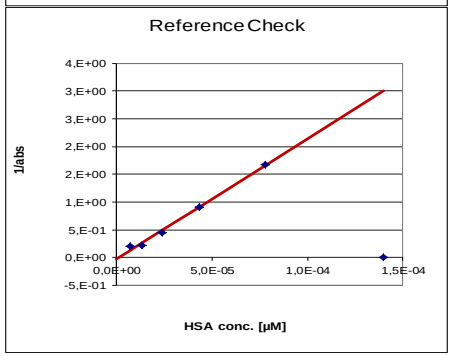
QC index: **7,1** **k_D= 9,24E-06** **fb=** **98,5% ± 4,531%**

warning messages: mismatch: estimated vs measured reference,

		consistency check					
signal < reference	4	0	1	1	1	1	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	1	1	0
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,00E-01	2,17E-01	4,55E-01	9,09E-01	1,67E+00	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	8,0%	56,0%	78,0%	88,0%	0,0%
(1-fu)/fu		0,000	0,087	1,273	3,545	7,333	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,087	1,273	3,545	7,333	0,000
slope		10328,79034					
intercept		1,51343					
r^2		0,0316					
prediction: (1-fu)/fu		1,6E+00	1,7E+00	1,8E+00	2,0E+00	2,3E+00	3,0E+00
outlier due to saturation and consistency		1	1	1	1	1	0
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
(1-fu)/fu		0,000	0,087	1,273	3,545	7,333	
slope		108181,66151					
intercept		-1,13828					
r^2		0,9948					
prediction		-0,337	0,304	1,459	3,536	7,276	
residuals		0,337	-0,218	-0,186	0,009	0,057	
standarized residuals		1,511	0,000	0,000	0,041	0,258	
Threshold		1,5					
statistical outlier	5	1	1	1	1	1	0
		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
		0,000	0,087	1,273	3,545	7,333	
slope		108181,66151	**9,24E-06**				
intercept		-1,13828					
r^2		0,9948					
prediction	-1,138	-0,337	0,304	1,459	3,536	7,276	14,007
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,40	2,80	3,90	4,40	0,00
free protein con. [µM]		7,41	12,94	21,21	39,31	73,38	140,00
free protein con. [M]	0	7,4E-06	1,3E-05	2,1E-05	3,9E-05	7,3E-05	1,4E-04
binding sites covered [%]		0,0%	3,0%	11,7%	9,0%	5,7%	0,0%
warning?							







reg	**9,24E-06**	**0,99477**	
avg	**4,66E-05**		1
choice	**9,24E-06**		

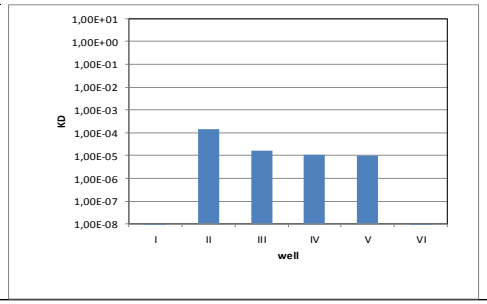
value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,00E-01	2,17E-01	4,55E-01	9,09E-01	1,67E+00	#WERT!
valid albumin conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
valid 1/signal		2,0E-01	2,2E-01	4,5E-01	9,1E-01	1,7E+00	
slope		21636,33230					
intercept		-0,02766					
r^2		0,9948					
predicted 1/signal	0	1,3E-01	2,6E-01	4,9E-01	9,1E-01	1,7E+00	3,0E+00
Predicted Reference Value		-36,16					
Actual Reference Value		5,00					
Relative deviation		823,2%					

				QC index: 7,1										warning messages
no. data points	9	reference	0	r^2 MA prediction	8	ref. Treatment	10	ref > signal	6	consistency	6			data points
		10,0%		0,9999		A		6		6				reference mismatch: estimat X
6		20,0%		0,999		M		5		5	6			ref. Treatment
5	9	50,0%		0,99	8	R	10	4	6	4				ref > signal references exceed X
4		100,0%		0,9	7	slopes	10	3		3				consistency data not consisten X
3		200,0%		0,8	6	1/abs > 0	5	2		2				r^2 MA prediction
2		500,0%		0,7	5	c(lipid)/c(buffer) >0	10	1		intercept				slopes
1		<500%	0	0,6	4			0		-1,14	5			intercept
0				0,5	3									binding
				<0.5										summary mismatch: estimated vs measured reference,
														references exceed signal: solubility problem? data not conisistent

		1. step outlier detection														
mean x		0,000		n	5											
$(x_i\text{-mean}(x))^2$		6,6E-10	3,9E-10	8,4E-11	1,0E-10	2,0E-09		initial r2:		0,9948						
$S(x_i\text{-mean}(x))^2$		3,2E-09						skip outlier detection if r2 >		0,98						
		0,60	0,68	0,77	0,77	0,18				omit	outlier detection					
residuals		0,337	-0,218	-0,186	0,009	0,057										
		0,0497						check, if estimation of reference assay volume becomes worse after outlier detection								
		0,0378	0,0158	0,0115	0,0000	0,0011										
s^2		0,0662								checking effect of outlier removal on intercept estimation of nt/c						
studentized residual		1,70	-1,03	-0,82	0,04	0,52				1	1	1	1	1	0	
		1,70	1,03	0,82	0,04	0,52		albumin conc. [µM]		0,000	0,000	0,000	0,000	0,000		
		1	1	1	1	1	0	(1-fu)/fu		0,0	0,087	1,273	3,545	7,333		
		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05			108181,66151	w/o outlier		with outlier				
		0,000	0,087	1,273	3,545	7,333			-1,13828		241,138	241,138	0,00	ok		
slope		108181,66151							0,9948	improvement?		no	1,0			
intercept		-1,13828								accept outlier:		**na**				
r^2		0,9948						abs(studentized residuals)		1,70	1,03	0,82	0,04	0,52		1,70
		2. step outlier detection									1,33364E-05	2,40055E-05	4,32099E-05	7,77778E-05		
mean x		0,000		n	5						0,1	1,3	3,5	7,3		
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00	0,00										
$S(x_i\text{-mean}(x))^2$		0,00														
		0,60	0,68	0,77	0,77	0,18										
residuals		0,337	-0,218	-0,186	0,009	0,057		second tier r2:		0,9948						
		0,0378	0,0158	0,0115	0,0000	0,0011		skip outlier detection if r2 >		0,98						
s^2		0,0662								omit	outlier detection					
studentized residual		1,70	-1,03	-0,82	0,04	0,52										
		1,70	1,03	0,82	0,04	0,52		no outliers will be recorded if there are only 3 or less points left at step 1 or 2								
		1	1	1	1	1	0									
		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05										
		0,000	0,087	1,273	3,545	7,333										
slope		108181,66151	**9,24E-06**													
intercept		-1,13828														
r^2		0,9948														

	Standard Error					
	0	1	1	1	1	0
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,600	2,200	1,100	0,600	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	0,400	2,800	3,900	4,400	0,000
KD		1,5E-04	1,7E-05	1,1E-05	1,0E-05	
	4,7E-05					
SE(KD)	3,408E-05					
fb		0,798	0,972	0,981	0,983	
	93,4%					
SE(fb)	4,531%					
n	4					
	1,00E-12	1,49E-04	1,67E-05	1,11E-05	1,00E-05	1,00E-12



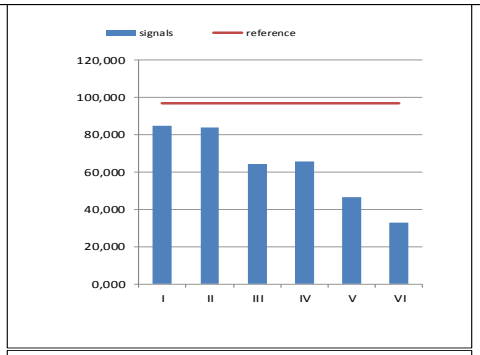
	test article No	10	**SL 9**				
	5,000 µM					0 nm / amu	
adj. Ref 1	99,000	A		av ref ok			
Ref 2	94,600	ref diff ok		max ref ok			
Signal Reference:	96,800	96,800	96,800	96,800	96,800	96,800	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	85,000	84,100	64,100	65,600	46,500	33,000	ok

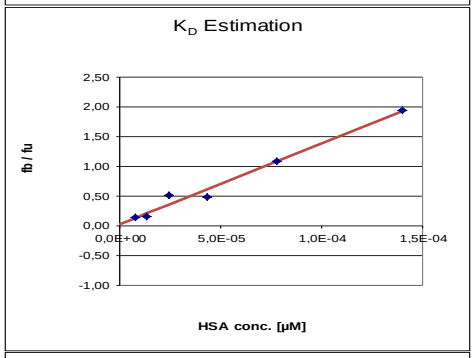
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

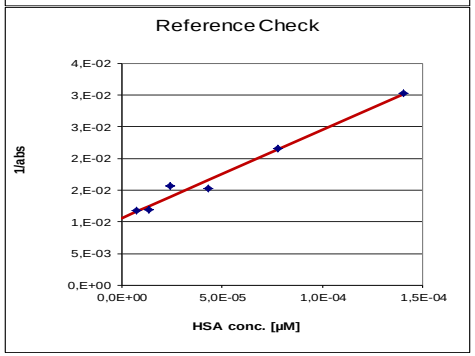
QC index: **9,5** **k_D= 7,41E-05** **fb=** **88,8% ± 1,005%** **r^2= 0,98015**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,18E-02	1,19E-02	1,56E-02	1,52E-02	2,15E-02	3,03E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		12,2%	13,1%	33,8%	32,2%	52,0%	65,9%
(1-fu)/fu		0,139	0,151	0,510	0,476	1,082	1,933
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,139	0,151	0,510	0,476	1,082	1,933
slope		13494,63548					
intercept		0,02747					
r^2		0,9801					
prediction: (1-fu)/fu		1,3E-01	2,1E-01	3,5E-01	6,1E-01	1,1E+00	1,9E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,139	0,151	0,510	0,476	1,082	1,933
slope		13494,63548					
intercept		0,02747					
r^2		0,9801					
prediction		0,127	0,207	0,351	0,611	1,077	1,917
residuals		0,011	-0,056	0,159	-0,135	0,005	0,017
standarized residuals		0,117	0,000	1,637	0,000	0,048	0,171
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
		0,139	0,151	0,510	0,476	1,082	1,933
slope		13494,63548	**7,41E-05**				
intercept		0,02747					
r^2		0,9801					
prediction	0,027	0,127	0,207	0,351	0,611	1,077	1,917
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,61	0,66	1,69	1,61	2,60	3,30
free protein con. [µM]		6,80	12,68	22,32	41,60	75,18	136,70
free protein con. [M]	0	6,8E-06	1,3E-05	2,2E-05	4,2E-05	7,5E-05	1,4E-04
binding sites covered [%]		8,2%	4,9%	7,0%	3,7%	3,3%	2,4%
warning?							







reg	**7,41E-05**	**0,98015**	
avg	**6,74E-05**		1
choice	**7,41E-05**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,18E-02	1,19E-02	1,56E-02	1,52E-02	2,15E-02	3,03E-02
valid albumin conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal		1,2E-02	1,2E-02	1,6E-02	1,5E-02	2,2E-02	3,0E-02
slope		139,40739					
intercept		0,01061					
r^2		0,9801					
predicted 1/signal	0	1,2E-02	1,2E-02	1,4E-02	1,7E-02	2,1E-02	3,0E-02
Predicted Reference Value		94,21					
Actual Reference Value		96,80					
Relative deviation		2,7%					

QC index: 9,5

no. data points	10	reference	10	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%	10	0,9999		A	10	6	10	6	10	data points	
6	10	20,0%	9	0,999		M		5		5		reference	
5		50,0%	8	0,99		R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		0,03	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,011	-0,056	0,159	-0,135	0,005	0,017
	0,0094					
	0,0000	0,0008	0,0063	0,0046	0,0000	0,0001
s^2	0,0118					
studentized residual	0,13	-0,61	1,66	-1,37	0,05	0,33
	0,13	0,61	1,66	1,37	0,05	0,33
	1	1	1	1	1	1
	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
	0,139	0,151	0,510	0,476	1,082	1,933
slope	13494,63548					
intercept	0,02747					
r^2	0,9801					
	2. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,011	-0,056	0,159	-0,135	0,005	0,017
	0,0000	0,0008	0,0063	0,0046	0,0000	0,0001
s^2	0,0118					
studentized residual	0,13	-0,61	1,66	-1,37	0,05	0,33
	0,13	0,61	1,66	1,37	0,05	0,33
	1	1	1	1	1	1
	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
	0,139	0,151	0,510	0,476	1,082	1,933
slope	13494,63548	**7,41E-05**				
intercept	0,02747					
r^2	0,9801					

initial r2: 0,9801

skip outlier detection if r2 > 0,98

omit outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1
albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000
(1-fu)/fu	0,1	0,151	0,510	0,476	1,082	1,933
	13494,63548 w/o outlier		with outlier			
	0,04236	239,958	239,973	0,00 ok		
	0,9801 improvement?		yes	1,0		
	accept outlier:		**na**			
abs(studentized residuals)	0,13	0,61	1,66	1,37	0,05	0,33	1,66
	7,4091E-06	1,33364E-05		4,32099E-05	7,77778E-05	0,00014	
	0,1	0,2		0,5	1,1	1,9	

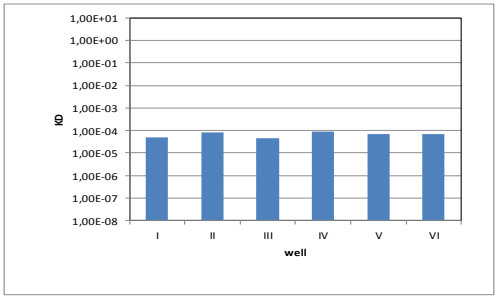
second tier r2: 0,9801

skip outlier detection if r2 > 0,98

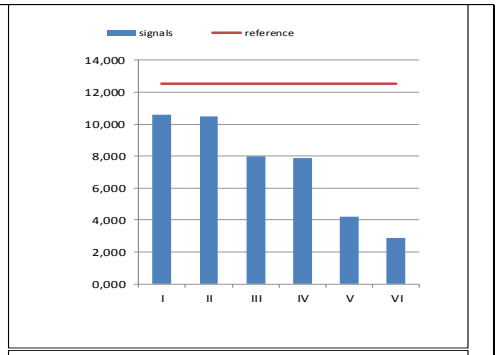
omit outlier detection

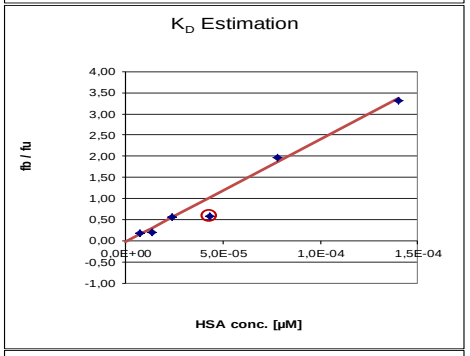
no outliers will be recorded if there are only 3 or less points left at step 1 or 2

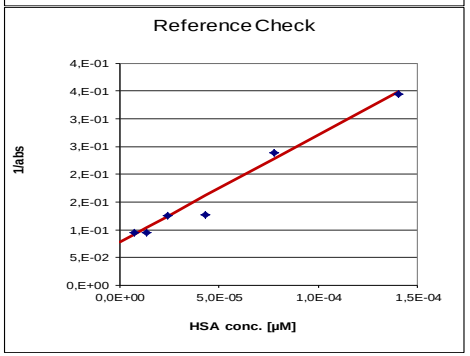
	Standard Error					
	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,390	4,344	3,311	3,388	2,402	1,705
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,610	0,656	1,689	1,612	2,598	3,295
KD	4,9E-05	8,4E-05	4,4E-05	8,7E-05	7,0E-05	7,1E-05
	6,7E-05					
SE(KD)	7,283E-06					
fb	0,923	0,875	0,931	0,871	0,894	0,893
	89,8%					
SE(fb)	1,005%					
n	6					
	4,90E-05	8,40E-05	4,37E-05	8,75E-05	6,95E-05	7,07E-05



	test article No	11		**SL 2**			
		5,000 µM				0 nm / amu	
adj. Ref 1		13,200	A		av ref ok		
Ref 2		11,800	ref diff ok		max ref ok		
Signal Reference:		12,500	12,500	12,500	12,500	12,500	12,500
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		10,600	10,500	8,000	7,900	4,200	2,900	ok
	-						
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		ok	ok	ok	**outlier**	ok	ok
							r^2= **0,99615**
QC index:	**9,6**	**k_D= 4,11E-05**		**fb=**	**93,5% ± 0,781%**		
warning messages:							
		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		9,43E-02	9,52E-02	1,25E-01	1,27E-01	2,38E-01	3,45E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		15,2%	16,0%	36,0%	36,8%	66,4%	76,8%
(1-fu)/fu		0,179	0,190	0,563	0,582	1,976	3,310
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,179	0,190	0,563	0,582	1,976	3,310
slope		24566,41552					
intercept		-0,11831					
r^2		0,9764					
prediction: (1-fu)/fu		6,4E-02	2,1E-01	4,7E-01	9,4E-01	1,8E+00	3,3E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,179	0,190	0,563	0,582	1,976	3,310
slope		24566,41552					
intercept		-0,11831					
r^2		0,9764					
prediction		0,064	0,209	0,471	0,943	1,792	3,321
residuals		0,116	-0,019	0,091	-0,361	0,184	-0,011
standarized residuals		0,599	0,000	0,472	0,000	0,952	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	0	1	1
		7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
		0,179	0,190	0,563		1,976	3,310
slope		24301,69626	**4,11E-05**				
intercept		-0,03223					
r^2		0,9961					
prediction	-0,032	0,148	0,292	0,551	1,018	1,858	3,370
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	0,582	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,76	0,80	1,80	1,84	3,32	3,84
free protein con. [µM]		6,65	12,54	22,21	41,37	74,46	136,16
free protein con. [M]	0	6,6E-06	1,3E-05	2,2E-05	4,1E-05	7,4E-05	1,4E-04
binding sites covered [%]		10,3%	6,0%	7,5%	4,3%	4,3%	2,7%
warning?							







reg	**4,11E-05**	**0,99615**	
avg	**4,42E-05**		1
choice	**4,11E-05**		

value of "-5.0" places outlier markers below visible chart area

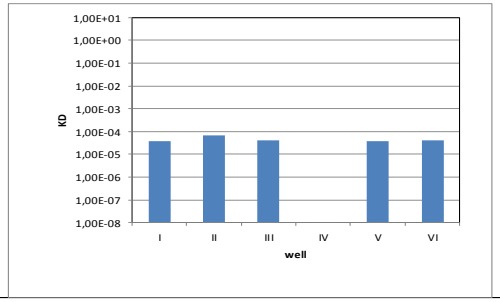
		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		9,43E-02	9,52E-02	1,25E-01	1,27E-01	2,38E-01	3,45E-01
valid albumin conc. [µM]		7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
valid 1/signal		9,4E-02	9,5E-02	1,3E-01		2,4E-01	3,4E-01
slope		1944,13570					
intercept		0,07742					
r^2		0,9961					
predicted 1/signal	0	9,2E-02	1,0E-01	1,2E-01	1,6E-01	2,3E-01	3,5E-01
Predicted Reference Value		12,92					
Actual Reference Value		12,50					
Relative deviation		3,2%					

QC index: 9,6

no. data points	9	reference	10	r^2 MA prediction	8	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%	10	0,9999		A	10	6	10	6	10	data points	
6		20,0%	9	0,999		M		5		5		reference	
5	9	50,0%	8	0,99	8	R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		-0,03	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,116	-0,019	0,091	-0,361	0,184	-0,011
	0,0372					
	0,0033	0,0001	0,0021	0,0326	0,0084	0,0000
s^2	0,0465					
studentized residual	0,65	-0,10	0,48	-1,84	0,97	-0,11
	0,65	0,10	0,48	1,84	0,97	0,11
	1	1	1	0	1	1
	7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
	0,179	0,190	0,563		1,976	3,310
slope	24301,69626					
intercept	-0,03223					
r^2	0,9961					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00		0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,64	0,68	0,74		0,75	0,20
residuals	0,031	-0,101	0,011		0,118	-0,060
	0,0003	0,0034	0,0000		0,0047	0,0012
s^2	0,0096					
studentized residual	0,40	-1,25	0,13		1,39	-1,37
	0,40	1,25	0,13		1,39	1,37
	1	1	1	0	1	1
	7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
	0,179	0,190	0,563		1,976	3,310
slope	24301,69626	**4,11E-05**				
intercept	-0,03223					
r^2	0,9961					

	Standard Error					
	1	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,240	4,200	3,200	3,160	1,680	1,160
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,760	0,800	1,800	1,840	3,320	3,840
KD	3,7E-05	6,6E-05	3,9E-05		3,8E-05	4,1E-05
	4,4E-05					
SE(KD)	5,441E-06					
fb	0,941	0,899	0,937		0,940	0,935
	93,0%					
SE(fb)	0,781%					
n	5					
	3,71E-05	6,58E-05	3,95E-05	1,00E-12	3,77E-05	4,11E-05



initial r2:		0,9764					
skip outlier detection if r2 >		0,98					
		consider	outlier detection				
check, if estimation of reference assay volume becomes worse after outlier detection							
		checking effect of outlier removal on intercept estimation of nt/c					
		1	1	1	0	1	1
albumin conc. [µM]		0,000	0,000	0,000		0,000	0,000
(1-fu)/fu		0,2	0,190	0,563		1,976	3,310
		24301,69626	w/o outlier	with outlier			
		-0,08169	240,082	240,118	0,00 ok		
		0,9961	improvement?	yes	6,1		
			accept outlier:	**yes**			
abs(studentized residuals)		0,65	0,10	0,48		0,97	0,11	0,97
		7,4091E-06	1,33364E-05	2,40055E-05		7,77778E-05	0,00014	
		0,2	0,2	0,6			3,3	
second tier r2:		0,9961					
skip outlier detection if r2 >		0,98					
		omit	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2							

test article No 12 **SL 3**

	5,000 µM					0 nm / amu	
adj. Ref 1	1,250	R		av ref too low			
Ref 2	1,400	ref diff ok		max ref too low R			
Signal Reference:	1,500	1,500	1,500	1,500	1,500	1,500	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	1,500	1,800	1,370	0,900	0,750	na	ok

-

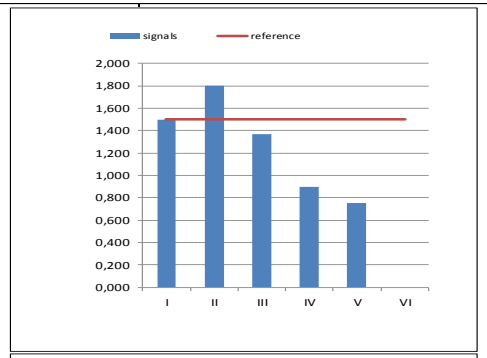
	missing values					
	1	1	1	1	1	0
	outlier detection					
consistency	ok	**inconsistent**	ok	ok	ok	**inconsistent**
saturation	**reference**	**na**	ok	ok	ok	**na**
statistics	ok	**na**	ok	ok	ok	**na**

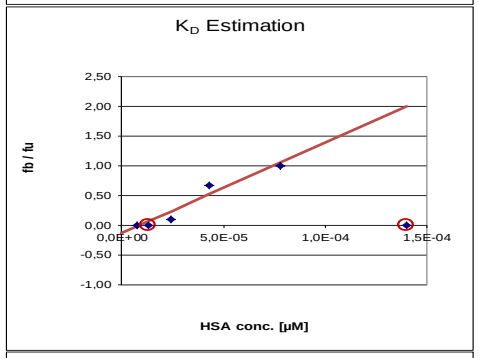
r^2= **0,93819**

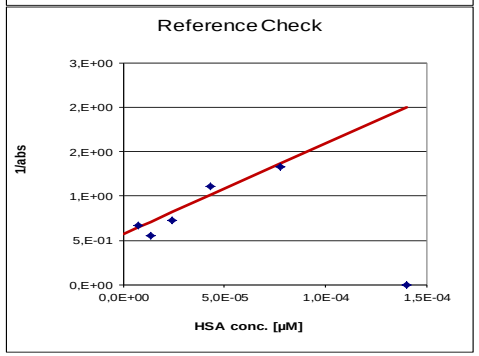
QC index: **7,2** **k_D= 6,57E-05** **fb=** **90,0% ± 6,445%**

warning messages: references exceed signal: solubility problem? data not consistent,

		consistency check					
signal < reference	3	0	0	1	1	1	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	4	1	0	1	1	1	0
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,67E-01	5,56E-01	7,30E-01	1,11E+00	1,33E+00	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	0,0%	8,7%	40,0%	50,0%	0,0%
(1-fu)/fu		0,000	0,000	0,095	0,667	1,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,000	0,095	0,667	1,000	0,000
slope		1498,45942					
intercept		0,21724					
r^2		0,0306					
prediction: (1-fu)/fu		2,3E-01	2,4E-01	2,5E-01	2,8E-01	3,3E-01	4,3E-01
outlier due to saturation and consistency		1	0	1	1	1	0
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06		2,4E-05	4,3E-05	7,8E-05	
(1-fu)/fu		0,000		0,095	0,667	1,000	
slope		15230,05534					
intercept		-0,13988					
r^2		0,9382					
prediction		-0,027		0,226	0,518	1,045	
residuals		0,027		-0,131	0,148	-0,045	
standarized residuals		0,229		0,000	1,256	0,000	
Threshold		1,5					
statistical outlier	4	1	0	1	1	1	0
		7,4E-06		2,4E-05	4,3E-05	7,8E-05	
		0,000		0,095	0,667	1,000	
slope		15230,05534	**6,57E-05**				
intercept		-0,13988					
r^2		0,9382					
prediction	-0,140	-0,027	0,063	0,226	0,518	1,045	1,992
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	0,000	-5,000	-5,000	-5,000	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,00	0,43	2,00	2,50	0,00
free protein con. [µM]		7,41	13,34	23,57	41,21	75,28	140,00
free protein con. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,1E-05	7,5E-05	1,4E-04
binding sites covered [%]		0,0%	0,0%	1,8%	4,6%	3,2%	0,0%
warning?							







reg	**6,57E-05**	**0,93819**
avg	**1,29E-04**	1
choice	**6,57E-05**	

value of "-5.0" places outlier markers below visible chart area

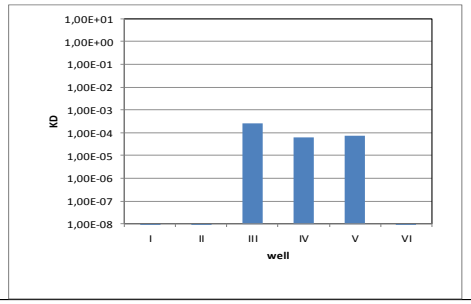
		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,67E-01	5,56E-01	7,30E-01	1,11E+00	1,33E+00	#WERT!
valid albumin conc. [µM]		7,4E-06		2,4E-05	4,3E-05	7,8E-05	
valid 1/signal		6,7E-01		7,3E-01	1,1E+00	1,3E+00	
slope		10153,37023					
intercept		0,57341					
r^2		0,9382					
predicted 1/signal	1	6,5E-01	7,1E-01	8,2E-01	1,0E+00	1,4E+00	2,0E+00
Predicted Reference Value		1,74					
Actual Reference Value		1,50					
Relative deviation		14,0%					

QC index: 7,2

no. data points	6	reference	9	r^2 MA predict	7	ref. Treatment	10	ref > signal	4	consistency	3		warning messages					
		10,0%		0,9999		A		6		6			data points					
6		20,0%	9	0,999		M		5		5			reference					
5		50,0%	8	0,99		R	10	4		4	3		ref. Treatment					
4	6	100,0%	7	0,9	7	slopes	10	3	4	3			ref > signal	references exceed	X			
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			consistency	data not consisten	X			
2		500,0%	3	0,7	5	id)/c(buffer) >0	10	1		intercept			r^2 MA prediction					
1		<500%		0,6	4			0		-0,14	10		slopes					
0				0,5	3								intercept					
				<0.5									binding					
													summary	references exceed signal: solubility problem? data not consisten				
													consistent					

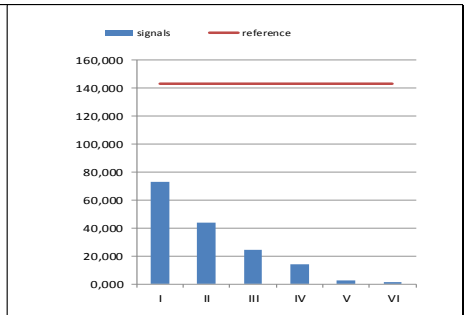
	1. step outlier detection					
mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	9,4E-10		2,0E-10	2,6E-11	1,6E-09	
$S(x_i\text{-mean}(x))^2$	2,7E-09					
	0,41		0,68	0,74	0,18	
residuals	0,027		-0,131	0,148	-0,045	
	0,0140					
	0,0004		0,0086	0,0110	0,0010	
s^2	0,0209					
studentized residual	0,29		-1,10	1,19	-0,74	
	0,29		1,10	1,19	0,74	
	1	0	1	1	1	0
	7,4E-06		2,4E-05	4,3E-05	7,8E-05	
	0,000		0,095	0,667	1,000	
slope	15230,05534					
intercept	-0,13988					
r^2	0,9382					
	2. step outlier detection					
mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	0,00		0,00	0,00	0,00	
$S(x_i\text{-mean}(x))^2$	0,00					
	0,41		0,68	0,74	0,18	
residuals	0,027		-0,131	0,148	-0,045	
	0,0004		0,0086	0,0110	0,0010	
s^2	0,0209					
studentized residual	0,29		-1,10	1,19	-0,74	
	0,29		1,10	1,19	0,74	
	1	0	1	1	1	0
	7,4E-06		2,4E-05	4,3E-05	7,8E-05	
	0,000		0,095	0,667	1,000	
slope	15230,05534	**6,57E-05**				
intercept	-0,13988					
r^2	0,9382					

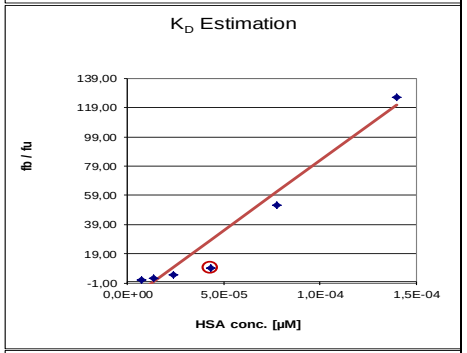
	Standard Error					
	0	0	1	1	1	0
	I	II	III	IV	V	VI
[A] in [M]	5,000	5,000	4,567	3,000	2,500	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	0,000	0,433	2,000	2,500	0,000
KD			2,5E-04	6,2E-05	7,5E-05	
	1,3E-04					
SE(KD)	6,008E-05					
fb			0,703	0,905	0,887	
	83,1%					
SE(fb)	6,445%					
n	3					
	1,00E-12	1,00E-12	2,48E-04	6,18E-05	7,53E-05	1,00E-12

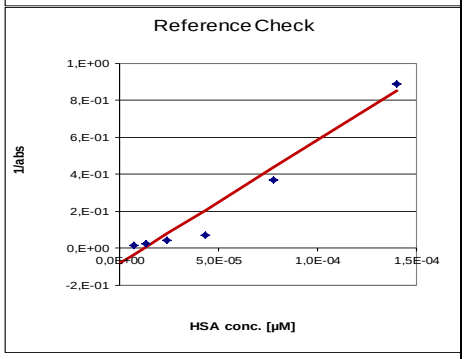


initial r2:	0,9382					
skip outlier detection if r2 >	0,98					
	consider	outlier detection				
check, if estimation of reference assay volume becomes worse after outlier detection						
		checking effect of outlier removal on intercept estimation of nt/c				
	1	0	1	1	1	0
albumin conc. [µM]	0,000		0,000	0,000	0,000	
(1-fu)/fu	0,0		0,095	0,667	1,000	
	15230,05534	w/o outlier	with outlier			
	-0,13988	240,140	240,140	0,00	ok	
	0,9382	improvement	no	1,0		
	accept outlier	**na**				
abs(studentized residuals)	0,29		1,10	1,19	0,74	1,19
	7,4091E-06		2,40055E-05	4,32099E-05	7,77778E-05	
	0,0		0,1		1,0	
second tier r2:	0,9382					
skip outlier detection if r2 >	0,98					
	consider	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2						

		test article No	13		**SL 13**			
		5,000 µM					0 nm / amu	
adj. Ref 1		87,700	M		av ref ok			
Ref 2		143,000	high ref diff	M	max ref ok			
Signal Reference:		143,000	143,000	143,000	143,000	143,000	143,000	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		72,700	43,800	24,500	13,900	2,700	1,125	ok
		Ref 1 <> Ref 2						
		missing values						
		1	1	1	1	1	1	
		outlier detection						
consistency		ok	ok	ok	ok	ok	ok	
saturation		ok	ok	ok	ok	ok	ok	
statistics		ok	ok	ok	**outlier**	ok	ok	
							r^2= **0,98328**	
QC index:	**7,4**	**k_D= 1,05E-06**		**fb=**	**99,8% ± 0,139%**			
warning messages:	poor intercept fit,							
		consistency check						
signal < reference	6	1	1	1	1	1	1	
binding > 0%		1	1	1	1	1	1	
steady increase in binding & summary	6	1	1	1	1	1	1	
		removal of outliers due to saturation						
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04	
1/signal in TRANSIL well		1,38E-02	2,28E-02	4,08E-02	7,19E-02	3,70E-01	8,89E-01	
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2	
fb in assay		49,2%	69,4%	82,9%	90,3%	98,1%	99,2%	
(1-fu)/fu		0,967	2,265	4,837	9,288	51,963	126,111	
saturation threshold		99,999%						
systematic outlier (saturation)		1	1	1	1	1	1	
consistent saturation		1	1	1	1	1	1	
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04	
(1-fu)/fu		0,967	2,265	4,837	9,288	51,963	126,111	
slope		964486,55294						
intercept		-16,57506						
r^2		0,9597						
prediction: (1-fu)/fu		-9,4E+00	-3,7E+00	6,6E+00	2,5E+01	5,8E+01	1,2E+02	
outlier due to saturation and consistency		1	1	1	1	1	1	
		statistical outlier detection & removal						
albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000	
(1-fu)/fu		0,967	2,265	4,837	9,288	51,963	126,111	
slope		964486,55294						
intercept		-16,57506						
r^2		0,9597						
prediction		-9,429	-3,712	6,578	25,100	58,441	118,453	
residuals		10,396	5,977	-1,741	-15,813	-6,478	7,658	
standarized residuals		1,042	0,599	0,000	0,000	0,000	0,768	
Threshold		1,5						
statistical outlier	5	1	1	1	0	1	1	
		7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04	
		0,967	2,265	4,837		51,963	126,111	
slope		952888,82777	**1,05E-06**					
intercept		-12,80361						
r^2		0,9833						
prediction	-12,804	-5,744	-0,096	10,071	28,371	61,310	120,601	
		outlier						
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04	
(1-fu)/fu		-5,000	-5,000	-5,000	9,288	-5,000	-5,000	
		Binding QC						
no. of binding sites		1						
conc. protein-compound complex [µM]		2,46	3,47	4,14	4,51	4,91	4,96	
free protein con. [µM]		4,95	9,87	19,86	38,70	72,87	135,04	
free protein con. [M]	0	5,0E-06	9,9E-06	2,0E-05	3,9E-05	7,3E-05	1,4E-04	
binding sites covered [%]		33,2%	26,0%	17,3%	10,4%	6,3%	3,5%	
warning?								







reg	**1,05E-06**	**0,98328**	
avg	**3,21E-06**		1
choice	**1,05E-06**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,38E-02	2,28E-02	4,08E-02	7,19E-02	3,70E-01	8,89E-01
valid albumin conc. [µM]		7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
valid 1/signal		1,4E-02	2,3E-02	4,1E-02		3,7E-01	8,9E-01
slope		6663,55824					
intercept		-0,08254					
r^2		0,9833					
predicted 1/signal	0	-3,3E-02	6,3E-03	7,7E-02	2,1E-01	4,4E-01	8,5E-01
Predicted Reference Value		-12,11					
Actual Reference Value		143,00					
Relative deviation		108,5%					

QC index: 7,4

no. data points	9	reference	5	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10	warning messages		
		10,0%		0,9999		A		6	10	6	10	data points		
6		20,0%		0,999		M	10	5		5		reference		
5	9	50,0%		0,99		R		4		4		ref. Treatment		
4		100,0%		0,9	7	slopes	10	3		3		ref > signal		
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency		
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction		
1		<500%		0,6	4			0		-12,80	0	slopes		
0				0,5	3							intercept	poor interce	X
				<0.5								binding		
												summary	poor intercept fit,	

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	10,396	5,977	-1,741	-15,813	-6,478	7,658
	99,4954					
	27,0196	8,9316	0,7579	62,5089	10,4898	14,6615
s^2	124,3692					
studentized residual	1,13	0,63	-0,18	-1,56	-0,66	1,49
	1,13	0,63	0,18	1,56	0,66	1,49
	1	1	1	0	1	1
	7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
	0,967	2,265	4,837		51,963	126,111
slope	952888,82777					
intercept	-12,80361					
r^2	0,9833					

initial r2: 0,9597

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	0	1	1
albumin conc. [µM]	0,000	0,000	0,000		0,000	0,000
(1-fu)/fu	1,0	2,265	4,837		51,963	126,111
	952888,82777	w/o outlier	with outlier			
	-8,23594	248,236	256,575	-0,03 ok		
	0,9833	improvement? yes		2,4		
		accept outlier: **yes**				
abs(studentized residuals)	1,13	0,63	0,18		0,66	1,49	1,13
	7,4091E-06	1,33364E-05	2,40055E-05		7,77778E-05	0,00014
		2,3	4,8		52,0	126,1

2. step outlier detection

mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00		0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,64	0,68	0,74		0,75	0,20
residuals	6,711	2,360	-5,234		-9,347	5,510
	15,0105	1,8571	9,1323		29,1221	10,1211
s^2	65,2432					
studentized residual	1,04	0,35	-0,76		-1,34	1,54
	1,04	0,35	0,76		1,34	1,54
	1	1	1	0	1	1
	7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
	0,967	2,265	4,837		51,963	126,111
slope	952888,82777	**1,05E-06**				
intercept	-12,80361					
r^2	0,9833					

second tier r2: 0,9833

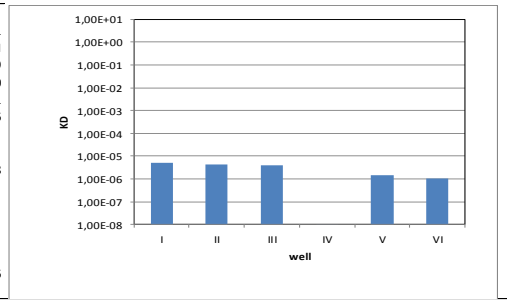
skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	2,542	1,531	0,857	0,486	0,094	0,039
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,458	3,469	4,143	4,514	4,906	4,961
KD	5,1E-06	4,4E-06	4,1E-06		1,4E-06	1,1E-06
	3,2E-06					
SE(KD)	8,250E-07					
fb	0,991	0,993	0,993		0,998	0,998
	99,5%					
SE(fb)	0,139%					
n	5					
	5,12E-06	4,36E-06	4,11E-06	1,00E-12	1,40E-06	1,07E-06



test article No 14 **SL 11**

		5,000 µM				0 nm / amu	
adj. Ref 1		143,200	M		av ref too low		
Ref 2		140,500	ref diff ok	M	max ref ok		
Signal Reference:		143,200	143,200	143,200	143,200	143,200	143,200
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		143,200	131,800	1149,000	103,000	79,800	45,200	ok

-

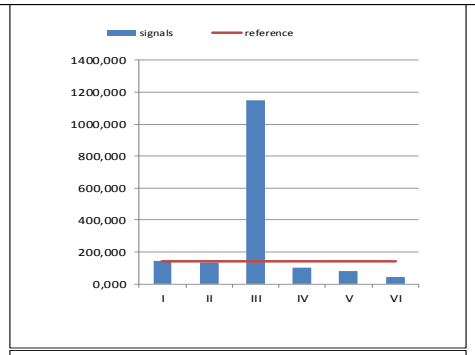
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	**inconsistent**	ok	ok	ok
saturation	ok	ok	**na**	ok	ok	ok
statistics	ok	ok	**na**	ok	ok	ok

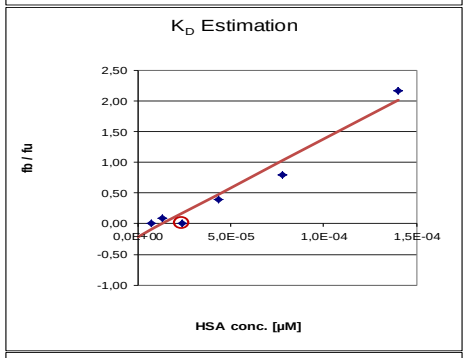
r^2= **0,96796**

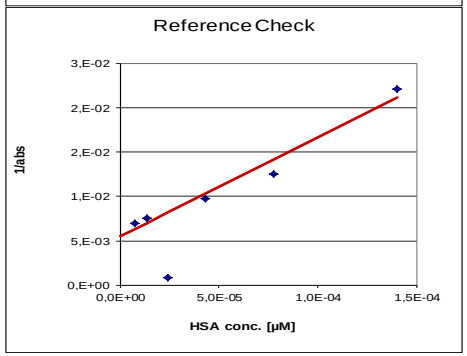
QC index: **8,2** **k_D= 6,26E-05** **fb=** **90,4% ± 1,957%**

warning messages: references exceed signal: solubility problem? data not consistent,

		consistency check					
signal < reference	4	0	1	0	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	0	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,98E-03	7,59E-03	8,70E-04	9,71E-03	1,25E-02	2,21E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	8,0%	0,0%	28,1%	44,3%	68,4%
(1-fu)/fu		0,000	0,086	0,000	0,390	0,794	2,168
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,086	0,000	0,390	0,794	2,168
slope		16325,79340					
intercept		-0,25867					
r^2		0,9651					
prediction: (1-fu)/fu		-1,4E-01	-4,1E-02	1,3E-01	4,5E-01	1,0E+00	2,0E+00
outlier due to saturation and consistency		1	1	0	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,000	0,086		0,390	0,794	2,168
slope		15962,90431					
intercept		-0,21157					
r^2		0,9680					
prediction		-0,093	0,001		0,478	1,030	2,023
residuals		0,093	0,085		-0,088	-0,235	0,145
standarized residuals		0,590	0,538		0,000	0,000	0,916
Threshold		1,5					
statistical outlier	5	1	1	0	1	1	1
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
		0,000	0,086		0,390	0,794	2,168
slope		15962,90431	**6,26E-05**				
intercept		-0,21157					
r^2		0,9680					
prediction	-0,212	-0,093	0,001	0,172	0,478	1,030	2,023
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	0,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,40	0,00	1,40	2,21	3,42
free protein con. [µM]		7,41	12,94	24,01	41,81	75,56	136,58
free protein con. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,2E-05	7,6E-05	1,4E-04
binding sites covered [%]		0,0%	3,0%	0,0%	3,2%	2,8%	2,4%
warning?							







reg	**6,26E-05**	**0,96796**	
avg	**1,04E-04**		1
choice	**6,26E-05**		

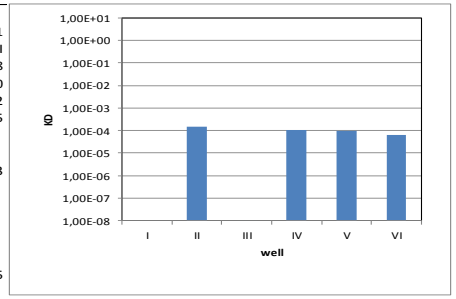
value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,98E-03	7,59E-03	8,70E-04	9,71E-03	1,25E-02	2,21E-02
valid albumin conc. [µM]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
valid 1/signal		7,0E-03	7,6E-03		9,7E-03	1,3E-02	2,2E-02
slope		111,47280					
intercept		0,00551					
r^2		0,9680					
predicted 1/signal	0	6,3E-03	7,0E-03	8,2E-03	1,0E-02	1,4E-02	2,1E-02
Predicted Reference Value		181,63					
Actual Reference Value		143,20					
Relative deviation		21,2%					

				QC index: 8,2									warning messages	
no. data points	9	reference	8	r^2 MA prediction	7	ref. Treatment	10	ref > signal	6	consistency	6		data points	
		10,0%		0,9999		A		6		6			reference	
6		20,0%		0,999		M	10	5		5	6		ref. Treatment	
5	9	50,0%	8	0,99		R		4	6	4			ref > signal references exc	X
4		100,0%	7	0,9	7	slopes	10	3		3			consistency data not consi:	X
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			r^2 MA prediction	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept			slopes	
1		<500%		0,6	4			0		-0,21	10		intercept	
0				0,5	3								binding	
				<0.5									summary references exceed signal: solubility problem? data not c	
													data not consistent	

		1. step outlier detection														
mean x		0,000		n	5											
$(x_i\text{-mean}(x))^2$		2,4E-09	1,8E-09		1,7E-10	4,6E-10	7,0E-09		initial r2:		0,9680					
$S(x_i\text{-mean}(x))^2$		1,2E-08							skip outlier detection if r2 >		0,98					
		0,60	0,64		0,79	0,76	0,21				consider	outlier detection				
residuals		0,093	0,085		-0,088	-0,235	0,145									
		0,0250							check, if estimation of reference assay volume becomes worse after outlier detection							
		0,0029	0,0024		0,0026	0,0185	0,0070									
s^2		0,0334								checking effect of outlier removal on intercept estimation of nt/c						
studentized residual		0,66	0,58		-0,54	-1,48	1,73			1	1	0	1	1	1	
		0,66	0,58		0,54	1,48	1,73		albumin conc. [µM]	0,000	0,000		0,000	0,000	0,000	
		1	1	0	1	1	1		(1-fu)/fu	0,0	0,086		0,390	0,794	2,168	
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04			15962,90431	w/o outlier	with outlier				
		0,000	0,086		0,390	0,794	2,168			-0,07611	240,076	240,212	0,00	ok		
slope		15962,90431								0,9680	improvement?	yes	1,0			
intercept		-0,21157									accept outlier:	**na**				
r^2		0,9680							abs(studentized residuals)	0,66	0,58		0,54	1,48	1,73	1,48
		2. step outlier detection								7,4091E-06	1,33364E-05		4,32099E-05	7,77778E-05	0,00014	
mean x		0,000		n	5					0,0	0,1		0,4		2,2	
$(x_i\text{-mean}(x))^2$		0,00	0,00		0,00	0,00	0,00									
$S(x_i\text{-mean}(x))^2$		0,00														
		0,60	0,64		0,79	0,76	0,21									
residuals		0,093	0,085		-0,088	-0,235	0,145		second tier r2:		0,9680					
		0,0029	0,0024		0,0026	0,0185	0,0070		skip outlier detection if r2 >		0,98					
s^2		0,0334									consider	outlier detection				
studentized residual		0,66	0,58		-0,54	-1,48	1,73									
		0,66	0,58		0,54	1,48	1,73		no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
		1	1	0	1	1	1									
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04									
		0,000	0,086		0,390	0,794	2,168									
slope		15962,90431	**6,26E-05**													
intercept		-0,21157														
r^2		0,9680														

		Standard Error				
	1	1	0	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,602	5,000	3,596	2,786	1,578
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	0,398	0,000	1,404	2,214	3,422
KD		1,5E-04		1,1E-04	9,5E-05	6,3E-05
	1,0E-04					
SE(KD)	1,602E-05					
fb		0,797		0,846	0,861	0,903
	85,2%					
SE(fb)	1,957%					
n	4					
		1,50E-04	1,00E-12	1,07E-04	9,51E-05	6,30E-05



test article No 15 **SL 10**

	5,000 µM					0 nm / amu	
adj. Ref 1	19,100	M		av ref ok			
Ref 2	5,500	high ref diff	M	max ref ok			
Signal Reference:	19,100	19,100	19,100	19,100	19,100	19,100	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	4,400	4,180	4,500	3,300	2,000	1,500	ok

Ref 1 <> Ref 2

	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	**inconsistent**	ok	ok	ok
saturation	ok	ok	**na**	ok	ok	ok
statistics	ok	ok	**na**	ok	ok	ok

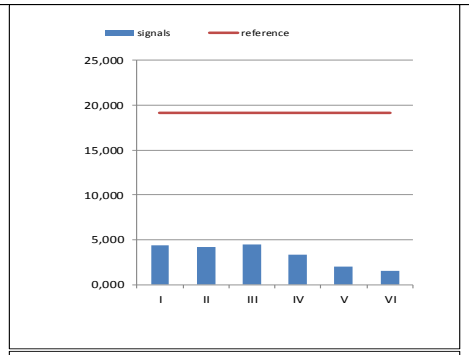
r^2= **0,97812**

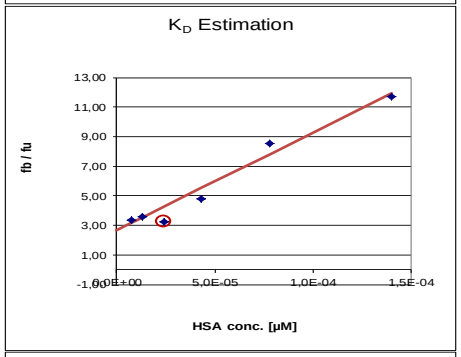
QC index: **6,9** **k_D= 1,51E-05** **fb=** **97,5% ± 0,327%**

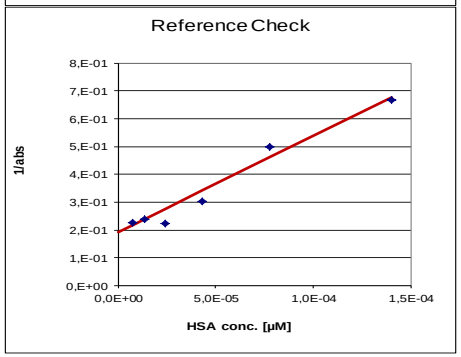
warning messages: data not consistent, poor intercept fit, invalid assumption [AP]<<[P]

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	0	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,27E-01	2,39E-01	2,22E-01	3,03E-01	5,00E-01	6,67E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		77,0%	78,1%	76,4%	82,7%	89,5%	92,1%
(1-fu)/fu		3,341	3,569	3,244	4,788	8,550	11,733
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		3,341	3,569	3,244	4,788	8,550	11,733
slope		68240,40492					
intercept		2,39370					
r^2		0,9680					
prediction: (1-fu)/fu		2,9E+00	3,3E+00	4,0E+00	5,3E+00	7,7E+00	1,2E+01
outlier due to saturation and consistency		1	1	0	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	0,000
(1-fu)/fu		3,341	3,569		4,788	8,550	11,733
slope		66095,85541					
intercept		2,67202					
r^2		0,9781					
prediction		3,162	3,554		5,528	7,813	11,925
residuals		0,179	0,016		-0,740	0,737	-0,192
standarized residuals		0,333	0,029		0,000	1,369	0,000
Threshold		1,5					
statistical outlier	5	1	1	0	1	1	1
		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
		3,341	3,569		4,788	8,550	11,733
slope		66095,85541	**1,51E-05**				
intercept		2,67202					
r^2		0,9781					
prediction	2,672	3,162	3,554	4,259	5,528	7,813	11,925
		outlier					
effective albumin conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	3,244	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		3,85	3,91	3,82	4,14	4,48	4,61
free protein con. [µM]		3,56	9,43	20,18	39,07	73,30	135,39
free protein con. [M]	0	3,6E-06	9,4E-06	2,0E-05	3,9E-05	7,3E-05	1,4E-04
binding sites covered [%]		51,9%	29,3%	15,9%	9,6%	5,8%	3,3%

warning? invalid assumption [AP]<<[P]







reg	**1,51E-05**	**0,97812**	
avg	**6,40E-06**		1
choice	**1,51E-05**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		2,27E-01	2,39E-01	2,22E-01	3,03E-01	5,00E-01	6,67E-01
valid albumin conc. [µM]		7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
valid 1/signal		2,3E-01	2,4E-01		3,0E-01	5,0E-01	6,7E-01
slope		3460,51599					
intercept		0,19225					
r^2		0,9781					
predicted 1/signal	0	2,2E-01	2,4E-01	2,8E-01	3,4E-01	4,6E-01	6,8E-01
Predicted Reference Value		5,20					
Actual Reference Value		19,10					
Relative deviation		72,8%					

QC index: 6,9

no. data points	9	reference	7	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	6
		10,0%		0,9999		A		6	10	6	
6		20,0%		0,999		M	10	5		5	6
5	9	50,0%		0,99		R		4		4	
4		100,0%	7	0,9	7	slopes	10	3		3	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept	
1		<500%		0,6	4			0		2,67	0
0				0,5	3						
				<0.5							

warning messages

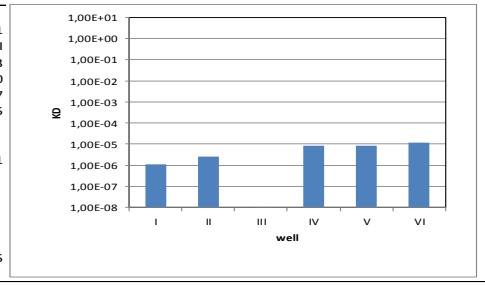
data points	
reference	
ref. Treatment	
ref > signal	
consistency	data not consi X
r^2 MA prediction	
slopes	
intercept	poor intercept X
binding	invalid assumpt X

summary data not consistent, poor intercept fit, invalid assumptio

assumption [AP]<<[P]

	1. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	2,4E-09	1,8E-09		1,7E-10	4,6E-10	7,0E-09
$S(x_i\text{-mean}(x))^2$	1,2E-08					
	0,60	0,64		0,79	0,76	0,21
residuals	0,179	0,016		-0,740	0,737	-0,192
	0,2901					
	0,0107	0,0001		0,1826	0,1811	0,0123
s^2	0,3868					
studentized residual	0,37	0,03		-1,34	1,36	-0,67
	0,37	0,03		1,34	1,36	0,67
	1	1	0	1	1	1
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
	3,341	3,569		4,788	8,550	11,733
slope	66095,85541					
intercept	2,67202					
r^2	0,9781					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00		0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,60	0,64		0,79	0,76	0,21
residuals	0,179	0,016		-0,740	0,737	-0,192
	0,0107	0,0001		0,1826	0,1811	0,0123
s^2	0,3868					
studentized residual	0,37	0,03		-1,34	1,36	-0,67
	0,37	0,03		1,34	1,36	0,67
	1	1	0	1	1	1
	7,4E-06	1,3E-05		4,3E-05	7,8E-05	1,4E-04
	3,341	3,569		4,788	8,550	11,733
slope	66095,85541	**1,51E-05**				
intercept	2,67202					
r^2	0,9781					

	Standard Error					
	1	1	0	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	1,152	1,094	1,178	0,864	0,524	0,393
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	3,848	3,906	3,822	4,136	4,476	4,607
KD	1,1E-06	2,6E-06		8,2E-06	8,6E-06	1,2E-05
	6,4E-06					
SE(KD)	1,960E-06					
fb	0,998	0,996		0,986	0,986	0,981
	98,9%					
SE(fb)	0,327%					
n	5					
	1,07E-06	2,64E-06	1,00E-12	8,16E-06	8,57E-06	1,15E-05



initial r2:		0,9781				
skip outlier detection if r2 >		0,98				
		consider	outlier detection			
check, if estimation of reference assay volume becomes worse after outlier detection						
checking effect of outlier removal on intercept estimation of nt/c						
	1	1	0	1	1	1
albumin conc. [µM]	0,000	0,000		0,000	0,000	0,000
(1-fu)/fu	3,3	3,569		4,788	8,550	11,733
	66095,85541	w/o outlier	with outlier			
	2,49243	237,508	237,328	0,00	ok	
	0,9781	improvement?	no	1,0		
		accept outlier:	**na**			
abs(studentized residuals)	0,37	0,03		1,34	1,36	0,67	1,36
	7,4091E-06	1,33364E-05		4,32099E-05	7,77778E-05	0,00014	
	3,3	3,6		4,8		11,7	
second tier r2:		0,9781					
skip outlier detection if r2 >		0,98					
		consider	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2							

4.3. AGP – Auswertung

TransilXL AGP Binding Kit

Version 2 Revision 00

required input
optional input

Assay Plate Parameters

Lot Number:	DESW
Date:	06.05.2013
Assay volume AGP:	240,0 µl
AGP concentration:	30,0 µM
α:	1,50

Quality Control Parameters

Saturation Threshold	99,9%	compound bound
Outlier Threshold	1,5	standard deviations
Threshold for outlier detection	0,98	r^2
Max difference of references:	30%	
MS saturation threshold	100000000	
maximum detectable K_d	0,0014	
model choice threshold	0,8	r^2

Model Parmeters

		maximum detectable Kd: > 0,0014
		fb HSA max: < 29,6%
1. Plasma protein binding		fb AGP max: < 1,4%
Physiological HSA conc.	5,88E-04	fb max: < 30,3%
Physiological AGP conc.	2,00E-05	fu min: < 69,7%

	Compound name	sample volume [µL]	stock conc. [µM]					
		15,0 µl	80,0 µM	Kd AGP	fb		r^2	TQI[1]
No 1	Doxorubicin	15,0 µl	80,0 µM	4,05E-04	4,7%	± 0,10	0,8191	8,8
No 2	SL 5	15,0 µl	80,0 µM	1,40E-03	< 1,4%	± 0,11	0,0487	4,6
No 3	SL 4	15,0 µl	80,0 µM	1,40E-03	< 1,4%	± 0,11	0,0736	2,8
No 4	SL 6	15,0 µl	80,0 µM	1,60E-04	11,1%	± 0,07	0,8857	8,8
No 5	SL 7	15,0 µl	80,0 µM	1,20E-06	94,3%	± 0,02	0,6037	6,4
No 6	SL 14	15,0 µl	80,0 µM	3,21E-06	86,2%	± 0,18	0,9859	7,3
No 7	SL 1	15,0 µl	80,0 µM	2,59E-04	7,2%	± 0,07	0,8572	7,9
No 8	SL 8	15,0 µl	80,0 µM	5,06E-06	79,8%	± 0,01	0,9826	9,4
No 9	SL 12	15,0 µl	80,0 µM	2,46E-05	44,9%	± 0,02	0,9968	9,6
No 10	SL 9	15,0 µl	80,0 µM	3,63E-06	84,6%	± 0,02	0,9885	8,4
No 11	SL 2	15,0 µl	80,0 µM	7,51E-05	21,0%	± 0,06	0,4327	7,8
No 12	SL 3	15,0 µl	80,0 µM	2,51E-05	44,4%	± 0,07	0,4811	8,1
No 13	SL 13	15,0 µl	80,0 µM	3,15E-05	38,8%	± 0,05	0,8500	8,4
No 14	SL 11	15,0 µl	80,0 µM	8,75E-05	18,6%	± 0,09	0,2045	6,7
No 15	SL 10	15,0 µl	80,0 µM	4,07E-06	83,1%	± 0,13	0,9749	8,0

ID	Compound name	K_d HSA	fu*
No 1	Doxorubicin	3,73E-04	38,1%
No 2	SL 5	NaN	-
No 3	SL 4	9,10E-06	1,5%
No 4	SL 6	4,25E-05	6,7%
No 5	SL 7	3,65E-05	3,0%
No 6	SL 14	3,19E-05	3,9%
No 7	SL 1	4,57E-05	7,2%
No 8	SL 8	3,14E-05	4,2%
No 9	SL 12	9,24E-06	1,5%
No 10	SL 9	7,41E-05	6,9%
No 11	SL 2	4,11E-05	6,4%
No 12	SL 3	6,57E-05	9,3%
No 13	SL 13	1,05E-06	0,2%
No 14	SL 11	6,26E-05	9,4%
No 15	SL 10	1,51E-05	2,2%

test article No 1 **Doxorubicin**

		5,000 µM				0 nm / amu	
adj. Ref 1		313,600	M		av ref too low		
Ref 2		0,000	high ref diff M		max ref ok		
Signal Reference:		313,600	313,600	313,600	313,600	313,600	313,600	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		253,000	270,100	256,000	237,500	250,000	241,500	ok

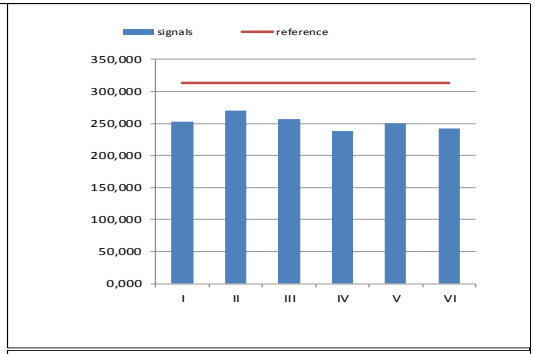
Ref 1 <> Ref 2

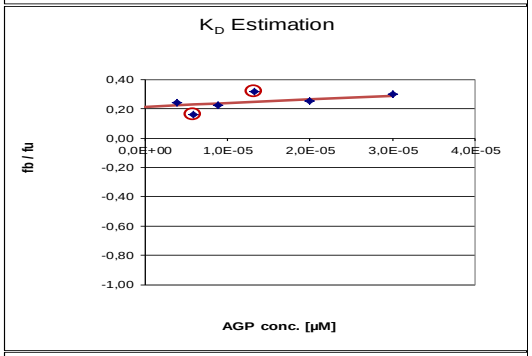
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	**outlier**	ok	**outlier**	ok	ok

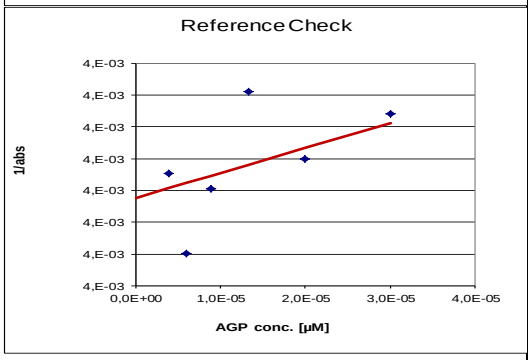
QC index: **8,8** **k_D= 4,05E-04** **fb=** **4,7% ± 10,068%** **r^2= 0,81909**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,95E-03	3,70E-03	3,91E-03	4,21E-03	4,00E-03	4,14E-03
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		19,3%	13,9%	18,4%	24,3%	20,3%	23,0%
(1-fu)/fu		0,240	0,161	0,225	0,320	0,254	0,299
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,240	0,161	0,225	0,320	0,254	0,299
slope		3526,09666					
intercept		0,20158					
r^2		0,3766					
prediction: (1-fu)/fu		2,2E-01	2,2E-01	2,3E-01	2,5E-01	2,7E-01	3,1E-01
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,240	0,161	0,225	0,320	0,254	0,299
slope		3526,09666					
intercept		0,20158					
r^2		0,3766					
prediction		0,216	0,222	0,233	0,249	0,272	0,307
residuals		0,024	-0,061	-0,008	0,072	-0,018	-0,009
standarized residuals		0,538	0,000	0,000	1,609	0,000	0,000
Threshold		1,5					
statistical outlier	4	1	0	1	0	1	1
		4,0E-06		8,9E-06		2,0E-05	3,0E-05
		0,240		0,225		0,254	0,299
slope		2470,10471	**4,05E-04**				
intercept		0,21556					
r^2		0,8191					
prediction	0,216	0,225	0,230	0,238	0,248	0,265	0,290
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	0,161	-5,000	0,320	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,97	0,69	0,92	1,21	1,01	1,15
free protein con. [µM]		2,98	5,23	7,97	12,12	18,99	28,85
free protein con. [M]	0	3,0E-06	5,2E-06	8,0E-06	1,2E-05	1,9E-05	2,9E-05
binding sites covered [%]		24,5%	11,7%	10,3%	9,1%	5,1%	3,8%
warning?							







reg	**4,05E-04**	**0,81909**	
avg	**5,48E-05**		1
choice	**4,05E-04**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,95E-03	3,70E-03	3,91E-03	4,21E-03	4,00E-03	4,14E-03
valid albumin conc. [µM]		4,0E-06		8,9E-06		2,0E-05	3,0E-05
valid 1/signal		4,0E-03		3,9E-03		4,0E-03	4,1E-03
slope		7,87661					
intercept		0,00388					
r^2		0,8191					
predicted 1/signal	0	3,9E-03	3,9E-03	3,9E-03	4,0E-03	4,0E-03	4,1E-03
Predicted Reference Value		257,99					
Actual Reference Value		313,60					
Relative deviation		17,7%					

QC index: 8,8

no. data points	6	reference	9	r^2 MA predict	6	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%		0,9999		A		6	10	6	10	data points	
6		20,0%	9	0,999		M	10	5		5		reference	
5		50,0%	8	0,99		R		4		4		ref. Treatment	
4	6	100,0%	7	0,9		slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		0,22	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

		1. step outlier detection					
mean x		0,000		n	6		
$(x_i\text{-mean}(x))^2$		9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$		4,8E-10					
		0,64	0,71	0,79	0,83	0,75	0,28
residuals		0,024	-0,061	-0,008	0,072	-0,018	-0,009
		0,0020					
		0,0001	0,0009	0,0000	0,0013	0,0001	0,0000
s^2		0,0025					
studentized residual		0,60	-1,46	-0,18	1,58	-0,41	-0,33
		0,60	1,46	0,18	1,58	0,41	0,33
		1	1	1	0	1	1
		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
		0,240	0,161	0,225		0,254	0,299
slope		3588,39160					
intercept		0,18635					
r^2		0,6231					
		2. step outlier detection					
mean x		0,000		n	5		
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00		0,00	0,00
$S(x_i\text{-mean}(x))^2$		0,00					
		0,60	0,67	0,75		0,72	0,25
residuals		0,039	-0,047	0,007		-0,004	0,005
		0,0005	0,0007	0,0000		0,0000	0,0000
s^2		0,0013					
studentized residual		1,42	-1,60	0,22		-0,12	0,25
		1,42	1,60	0,22		0,12	0,25
		1	0	1	0	1	1
		4,0E-06		8,9E-06		2,0E-05	3,0E-05
		0,240		0,225		0,254	0,299
slope		2470,10471	**4,05E-04**				
intercept		0,21556					
r^2		0,8191					

initial r2: 0,3766

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	0	1	1
albumin conc. [µM]	0,000	0,000	0,000		0,000	0,000
(1-fu)/fu	0,2	0,161	0,225		0,254	0,299
	3588,39160 w/o outlier		with outlier			
	0,19102	239,809	239,798	0,00 ok		
	0,6231 improvement?		no	1,7		
	accept outlier:		**yes**			
abs(studentized residuals)	0,60	1,46	0,18		0,41	0,33 1,46
	3,95062E-06	5,92593E-06	8,88889E-06		0,00002	0,00003
	0,2		0,2		0,3	0,3

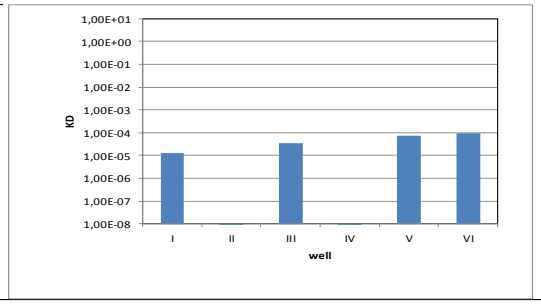
second tier r2: 0,6231

skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	1	0	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,034	4,306	4,082	3,787	3,986	3,850
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,966	0,694	0,918	1,213	1,014	1,150
KD	1,2E-05		3,5E-05		7,5E-05	9,7E-05
	5,5E-05					
SE(KD)	1,896E-05					
fb	0,616		0,361		0,211	0,171
	34,0%					
SE(fb)	10,068%					
n	4					
	1,25E-05	1,00E-12	3,54E-05	1,00E-12	7,46E-05	9,66E-05



test article No 2 **SL 5**

		5,000 µM				0 nm / amu	
adj. Ref 1		8,300	M		av ref ok		
Ref 2		5,600	high ref diff	M	max ref ok		
Signal Reference:		8,300	8,300	8,300	8,300	8,300	8,300
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		3,000	3,500	4,300	4,200	2,300	3,400 ok

Ref 1 <> Ref 2

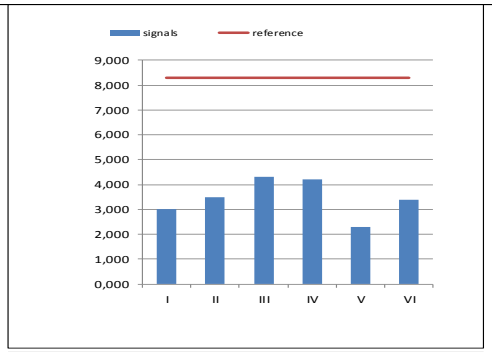
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	**inconsistent**	ok	ok	ok
saturation	ok	ok	**na**	ok	ok	ok
statistics	ok	ok	**na**	ok	**outlier**	ok

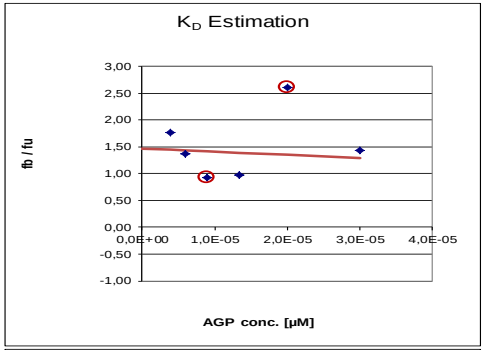
QC index: 4,6 **k_D= 1,40E-03** **fb= < 1,4% ± 10,842%** **r^2= 0,04867**

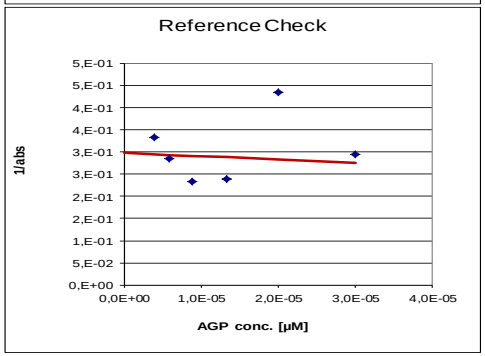
warning messages: data not consistent,

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	0	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,33E-01	2,86E-01	2,33E-01	2,38E-01	4,35E-01	2,94E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		63,9%	57,8%	48,2%	49,4%	72,3%	59,0%
(1-fu)/fu		1,767	1,371	0,930	0,976	2,609	1,441
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		1,767	1,371	0,930	0,976	2,609	1,441
slope		15203,08904					
intercept		1,30771					
r^2		0,0583					
prediction: (1-fu)/fu		1,4E+00	1,4E+00	1,4E+00	1,5E+00	1,6E+00	1,8E+00
outlier due to saturation and consistency		1	1	0	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06		1,3E-05	2,0E-05	0,000
(1-fu)/fu		1,767	1,371		0,976	2,609	1,441
slope		8743,75972					
intercept		1,50481					
r^2		0,0232					
prediction		1,539	1,557		1,621	1,680	1,767
residuals		0,227	-0,185		-0,645	0,929	-0,326
standarized residuals		0,375	0,000		0,000	1,532	0,000
Threshold		1,5					
statistical outlier	4	1	1	0	1	0	1
		4,0E-06	5,9E-06		1,3E-05		3,0E-05
		1,767	1,371		0,976		1,441
slope		-6046,86482	**-1,65E-04**				
intercept		1,46930					
r^2		0,0487					
prediction	1,469	1,445	1,433	1,416	1,389	1,348	1,288
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	0,930	-5,000	2,609	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		3,19	2,89	2,41	2,47	3,61	2,95
free protein con. [µM]		0,76	3,03	6,48	10,86	16,39	27,05
free protein con. [M]	0	7,6E-07	3,0E-06	6,5E-06	1,1E-05	1,6E-05	2,7E-05
binding sites covered [%]		80,8%	48,8%	27,1%	18,5%	18,1%	9,8%

warning? invalid assumption [AP]<<[P]







reg	**-1,65E-04**	**0,04867**
avg	**8,13E-06**	0
choice	**1,40E-03**	

value of "-5.0" places outlier markers below visible chart area

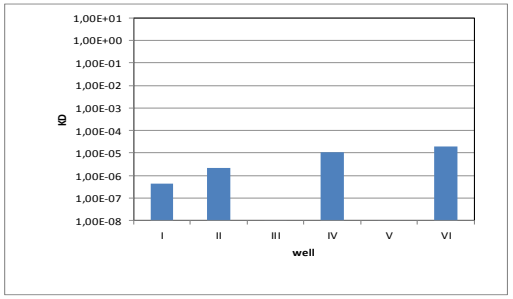
		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,33E-01	2,86E-01	2,33E-01	2,38E-01	4,35E-01	2,94E-01
valid albumin conc. [µM]		4,0E-06	5,9E-06		1,3E-05		3,0E-05
valid 1/signal		3,3E-01	2,9E-01		2,4E-01		2,9E-01
slope		-728,53793					
intercept		0,29751					
r^2		0,0487					
predicted 1/signal	0	2,9E-01	2,9E-01	2,9E-01	2,9E-01	2,8E-01	2,8E-01
Predicted Reference Value		3,36					
Actual Reference Value		8,30					
Relative deviation		59,5%					

QC index: 4,6

no. data points	6	reference	7	r^2 MA predict	0	ref. Treatment	10	ref > signal	10	consistency	6	warning messages						
		10,0%		0,9999		A		6	10	6		data points						
6		20,0%		0,999		M	10	5		5	6	reference						
5		50,0%		0,99		R		4		4		ref. Treatment						
4	6	100,0%	7	0,9		slopes	0	3		3		ref > signal						
3		200,0%	5	0,8		1/abs > 0	0	2		2		consistency	data not consisten	X				
2		500,0%	3	0,7		c(lipid)/c(buffer) >0	0	1		intercept		r^2	MA prediction poor model fit,	X				
1		<500%		0,6				0		1,47	5	slopes	slopes deviate fro	X				
0				0,5								intercept						
				<0.5	0							binding	invalid assumptior	X				
												summary	data not consistent,					

		1. step outlier detection										poor model fit, slopes deviate from model assumption				
mean x		0,000		n	5							invalid assumption [AP]<<[P]				
$(x_i\text{-mean}(x))^2$		1,1E-10	7,6E-11		1,7E-12	2,9E-11	2,4E-10		initial r2:		0,0232					
$S(x_i\text{-mean}(x))^2$		4,6E-10							skip outlier detection if r2 >		0,98					
		0,55	0,63		0,80	0,74	0,28				consider	outlier detection				
residuals		0,227	-0,185		-0,645	0,929	-0,326									
		0,3679							check, if estimation of reference assay volume becomes worse after outlier detection							
		0,0172	0,0114		0,1388	0,2877	0,0354									
s^2		0,4905								checking effect of outlier removal on intercept estimation of nt/c						
studentized residual		0,44	-0,33		-1,03	1,54	-0,87			1	1	0	1	0	1	
		0,44	0,33		1,03	1,54	0,87		albumin conc. [µM]	0,000	0,000		0,000		0,000	
		1	1	0	1	0	1		(1-fu)/fu	1,8	1,371		0,976		1,441	
		4,0E-06	5,9E-06		1,3E-05		3,0E-05			-6046,86482	w/o outlier	with outlier				
		1,767	1,371		0,976		1,441			1,95772	238,042	238,495	0,00	ok		
slope		-6046,86482								0,0487	improvement?	yes	1,0			
intercept		1,46930									accept outlier:	**yes**				
r^2		0,0487							abs(studentized residuals)	0,44	0,33		1,03		0,87	1,03
		2. step outlier detection								3,95062E-06	5,92593E-06		1,33333E-05		0,00003	
mean x		0,000		n	4					1,8	1,4				1,4	
$(x_i\text{-mean}(x))^2$		0,00	0,00		0,00		0,00									
$S(x_i\text{-mean}(x))^2$		0,00														
		0,54	0,62		0,75		0,09									
residuals		0,321	-0,062		-0,412		0,153		second tier r2:		0,0487					
		0,0516	0,0019		0,0851		0,0117		skip outlier detection if r2 >		0,98					
s^2		0,1503									consider	outlier detection				
studentized residual		1,13	-0,20		-1,23		1,34									
		1,13	0,20		1,23		1,34		no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
		1	1	0	1	0	1									
		4,0E-06	5,9E-06		1,3E-05		3,0E-05									
		1,767	1,371		0,976		1,441									
slope		-6046,86482	**-1,65E-04**													
intercept		1,46930														
r^2		0,0487														

		Standard Error				
		1	1	0	1	0	1
		I	II	III	IV	V	VI
[A] in [M]		1,807	2,108	2,590	2,530	1,386	2,048
[B] in [M]		3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]		3,193	2,892	2,410	2,470	3,614	2,952
KD		4,3E-07	2,2E-06		1,1E-05		1,9E-05
		8,1E-06					
SE(KD)		4,247E-06					
fb		0,979	0,900		0,642		0,516
		75,9%					
SE(fb)		10,842%					
n		4					
		4,29E-07	2,21E-06	1,00E-12	1,11E-05	1,00E-12	1,88E-05



test article No 3 **SL 4**

		5,000 µM				0 nm / amu	
adj. Ref 1		2,400	M		av ref ok		
Ref 2		3,800	high ref diff	M	max ref ok		
Signal Reference:		3,800	3,800	3,800	3,800	3,800	3,800
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		2,600	3,800	2,400	2,700	3,300	4,300	ok

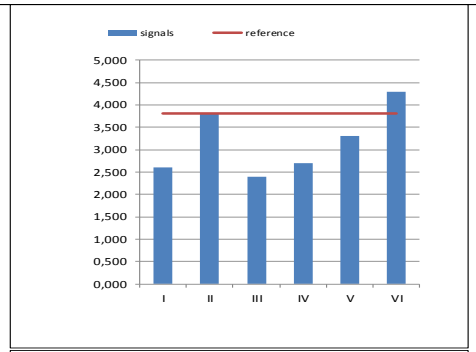
Ref 1 <> Ref 2

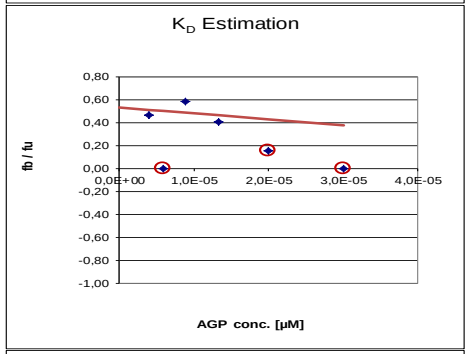
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	**inconsistent**	ok	ok	**inconsistent**	**inconsistent**
saturation	ok	**na**	ok	ok	**na**	**na**
statistics	ok	**na**	ok	ok	**na**	**na**

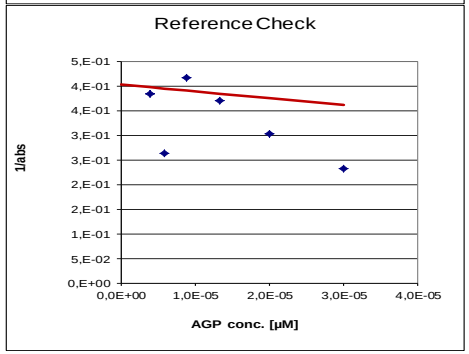
QC index: 2,8 **k_D= 1,40E-03** **fb= < 1,4% ± 11,250%** **r^2= 0,07359**

warning messages: few valid data points, references exceed signals

		consistency check					
signal < reference	4	1	0	1	1	1	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	3	1	0	1	1	0	0
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,85E-01	2,63E-01	4,17E-01	3,70E-01	3,03E-01	2,33E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		31,6%	0,0%	36,8%	28,9%	13,2%	0,0%
(1-fu)/fu		0,462	0,000	0,583	0,407	0,152	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,462	0,000	0,583	0,407	0,152	0,000
slope		-13371,93952					
intercept		0,45027					
r^2		0,2761					
prediction: (1-fu)/fu		4,0E-01	3,7E-01	3,3E-01	2,7E-01	1,8E-01	4,9E-02
outlier due to saturation and consistency		1	0	1	1	0	0
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06		8,9E-06	1,3E-05		
(1-fu)/fu		0,462		0,583	0,407		
slope		-5207,74198					
intercept		0,52953					
r^2		0,0736					
prediction		0,509		0,483	0,460		
residuals		-0,047		0,100	-0,053		
standarized residuals		0,000		1,154	0,000		
Threshold		1,5					
statistical outlier	3	1	0	1	1	0	0
		4,0E-06		8,9E-06	1,3E-05		
		0,462		0,583	0,407		
slope		-5207,74198	**-1,92E-04**				
intercept		0,52953					
r^2		0,0736					
prediction	0,530	0,509	0,499	0,483	0,460	0,425	0,373
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	0,000	-5,000	-5,000	0,152	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		1,58	0,00	1,84	1,45	0,66	0,00
free protein con. [µM]		2,37	5,93	7,05	11,89	19,34	30,00
free protein con. [M]	0	2,4E-06	5,9E-06	7,0E-06	1,2E-05	1,9E-05	3,0E-05
binding sites covered [%]		40,0%	0,0%	20,7%	10,9%	3,3%	0,0%
warning?							







reg	**-1,92E-04**	**0,07359**
avg	**1,55E-05**	0
choice	**1,40E-03**	

value of "-5.0" places outlier markers below visible chart area

		reference QC				
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,85E-01	2,63E-01	4,17E-01	3,70E-01	3,03E-01	2,33E-01
valid albumin conc. [µM]		4,0E-06		8,9E-06	1,3E-05		
valid 1/signal		3,8E-01		4,2E-01	3,7E-01		
slope		-1370,45842					
intercept		0,40251					
r^2		0,0736					
predicted 1/signal	0	4,0E-01	3,9E-01	3,9E-01	3,8E-01	3,8E-01	3,6E-01
Predicted Reference Value		2,48					
Actual Reference Value		3,80					
Relative deviation		34,6%					

QC index: 2,8

no. data points	1	reference	8	r^2 MA predict	0	ref. Treatment	10	ref > signal	6	consistency	1				warning messages			
		10,0%		0,9999		A		6		6					data points few valid data poi		X	
6		20,0%		0,999		M	10	5		5					reference			
5		50,0%	8	0,99		R		4	6	4					ref. Treatment			
4		100,0%	7	0,9		slopes	0	3		3	1				ref > signal references exceed		X	
3	1	200,0%	5	0,8		1/abs > 0	0	2		2					consistency data not consisten		X	
2		500,0%	3	0,7		c(lipid)/c(buffer) >0	0	1		intercept					r^2 MA prediction poor model fit,		X	
1		<500%		0,6				0		0,53	5				slopes slopes deviate fro		X	
0				0,5											intercept			
				<0.5	0										binding			
															summary few valid data points, references exceed signals			

		1. step outlier detection				
mean x		0,000		n	3	
$(x_i\text{-mean}(x))^2$		2,3E-11		2,7E-14	2,1E-11	
$S(x_i\text{-mean}(x))^2$		4,4E-11				
		0,15		0,67	0,18	
residuals		-0,047		0,100	-0,053	
		0,0075				
		0,0022		0,0100	0,0028	
s^2		0,0150				
studentized residual		-1,00		1,00	-1,00	
		1,00		1,00	1,00	
		1	0	1	1	0	0
		4,0E-06		8,9E-06	1,3E-05	
		0,462		0,583	0,407	
slope		-5207,74198				
intercept		0,52953				
r^2		0,0736				
		2. step outlier detection				
mean x		0,000		n	3	
$(x_i\text{-mean}(x))^2$		0,00		0,00	0,00	
$S(x_i\text{-mean}(x))^2$		0,00				
		0,15		0,67	0,18	
residuals		-0,047		0,100	-0,053	
		0,0022		0,0100	0,0028	
s^2		0,0150				
studentized residual		-1,00		1,00	-1,00	
		1,00		1,00	1,00	
		1	0	1	1	0	0
		4,0E-06		8,9E-06	1,3E-05	
		0,462		0,583	0,407	
slope		-5207,74198	**-1,92E-04**			
intercept		0,52953				
r^2		0,0736				

solubitiy problems? data not consistent, poor model fit,
slopes deviate from model assumption

initial r2:	0,0736
skip outlier detection if r2 >	0,98
	consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

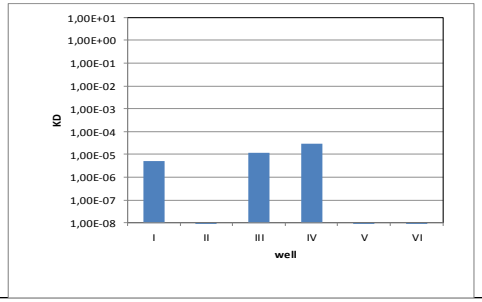
checking effect of outlier removal on intercept estimation of nt/c

	1	0	1	1	0	0
albumin conc. [µM]	0,000		0,000	0,000		
(1-fu)/fu	0,5		0,583	0,407		
	-5207,74198	w/o outlier	with outlier			
	0,52953	239,470	239,470	0,00 ok		
	0,0736	improvement:	no	1,0		
		accept outlier	**na**			
abs(studentized residuals)	1,00		1,00	1,00		1,00
	3,95062E-06		8,88889E-06	1,33333E-05		

second tier r2:	0,0736
skip outlier detection if r2 >	0,98
	consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

		Standard Error				
		1	0	1	1	0	0
		I	II	III	IV	V	VI
[A] in [M]		3,421	5,000	3,158	3,553	4,342	5,000
[B] in [M]		3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]		1,579	0,000	1,842	1,447	0,658	0,000
KD		5,1E-06		1,2E-05	2,9E-05		
		1,5E-05					
SE(KD)		7,142E-06					
fb		0,796		0,623	0,407		
		60,9%					
SE(fb)		11,250%					
n		3					
		5,14E-06	1,00E-12	1,21E-05	2,92E-05	1,00E-12	1,00E-12



test article No 4 **SL 6**

		5,000 µM				0 nm / amu	
adj. Ref 1		81,300	A		av ref ok		
Ref 2		70,500	ref diff ok		max ref ok		
Signal Reference:		75,900	75,900	75,900	75,900	75,900	75,900
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		70,000	58,000	59,900	51,800	55,500	52,900	ok

-

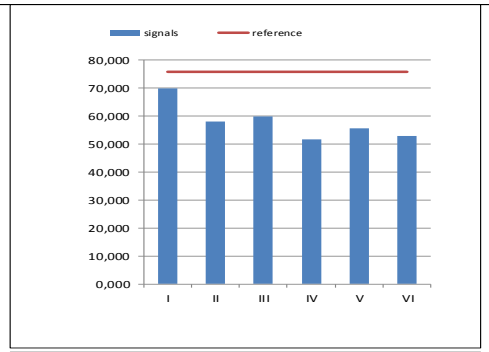
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		**outlier**	ok	ok	**outlier**	ok	ok

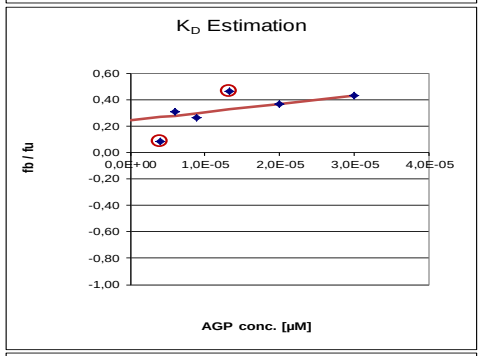
r^2= **0,88572**

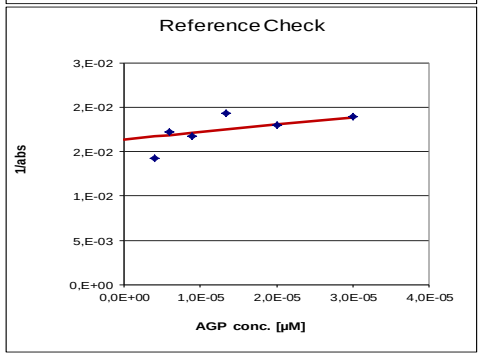
QC index: **8,8** **k_D= 1,60E-04** **fb=** **11,1% ± 7,390%**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		1,43E-02	1,72E-02	1,67E-02	1,93E-02	1,80E-02	1,89E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		7,8%	23,6%	21,1%	31,8%	26,9%	30,3%
(1-fu)/fu		0,084	0,309	0,267	0,465	0,368	0,435
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,084	0,309	0,267	0,465	0,368	0,435
slope		9828,61175					
intercept		0,18678					
r^2		0,4922					
prediction: (1-fu)/fu		2,3E-01	2,5E-01	2,7E-01	3,2E-01	3,8E-01	4,8E-01
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,084	0,309	0,267	0,465	0,368	0,435
slope		9828,61175					
intercept		0,18678					
r^2		0,4922					
prediction		0,226	0,245	0,274	0,318	0,383	0,482
residuals		-0,141	0,064	-0,007	0,147	-0,016	-0,047
standarized residuals		0,000	0,647	0,000	1,501	0,000	0,000
Threshold		1,5					
statistical outlier	4	0	1	1	0	1	1
			5,9E-06	8,9E-06		2,0E-05	3,0E-05
			0,309	0,267		0,368	0,435
slope		6232,60713	**1,60E-04**				
intercept		0,24353					
r^2		0,8857					
prediction	0,244	0,268	0,280	0,299	0,327	0,368	0,431
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,084	-5,000	-5,000	0,465	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,39	1,18	1,05	1,59	1,34	1,52
free protein con. [µM]		3,56	4,75	7,83	11,75	18,66	28,48
free protein con. [M]	0	3,6E-06	4,7E-06	7,8E-06	1,2E-05	1,9E-05	2,8E-05
binding sites covered [%]		9,8%	19,9%	11,9%	11,9%	6,7%	5,1%
warning?							







reg	**1,60E-04**	**0,88572**	
avg	**4,02E-05**		1
choice	**1,60E-04**		

value of "-5.0" places outlier markers below visible chart area

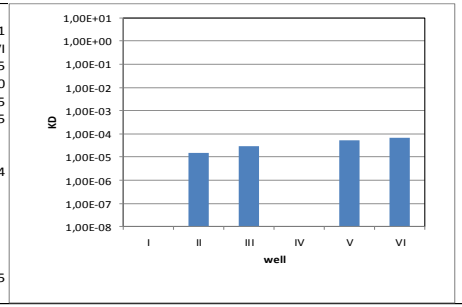
		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		1,43E-02	1,72E-02	1,67E-02	1,93E-02	1,80E-02	1,89E-02
valid albumin conc. [µM]			5,9E-06	8,9E-06		2,0E-05	3,0E-05
valid 1/signal			1,7E-02	1,7E-02		1,8E-02	1,9E-02
slope		82,11604					
intercept		0,01638					
r^2		0,8857					
predicted 1/signal	0	1,7E-02	1,7E-02	1,7E-02	1,7E-02	1,8E-02	1,9E-02
Predicted Reference Value		61,04					
Actual Reference Value		75,90					
Relative deviation		19,6%					

QC index: 8,8

no. data points	6	reference	9	r^2 MA predict	6	ref. Treatment	10	ref > signal	10	consistency	10		warning messages	
		10,0%		0,9999		A	10	6	10	6	10		data points	
6		20,0%	9	0,999		M		5		5			reference	
5		50,0%	8	0,99		R		4		4			ref. Treatment	
4	6	100,0%	7	0,9		slopes	10	3		3			ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept			r^2 MA prediction	
1		<500%		0,6	4			0		0,24	10		slopes	
0				0,5	3								intercept	
				<0.5									binding	
													summary	

		1. step outlier detection					
mean x		0,000		n	6		
$(x_i\text{-mean}(x))^2$		9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$		4,8E-10					
		0,64	0,71	0,79	0,83	0,75	0,28
residuals		-0,141	0,064	-0,007	0,147	-0,016	-0,047
		0,0096					
		0,0050	0,0010	0,0000	0,0054	0,0001	0,0005
s^2		0,0121					
studentized residual		-1,61	0,69	-0,07	1,47	-0,17	-0,80
		1,61	0,69	0,07	1,47	0,17	0,80
		0	1	1	1	1	1
			5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
			0,309	0,267	0,465	0,368	0,435
slope		5373,41661					
intercept		0,28468					
r^2		0,3873					
		2. step outlier detection					
mean x		0,000		n	5		
$(x_i\text{-mean}(x))^2$			0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$		0,00					
			0,55	0,68	0,79	0,75	0,24
residuals			-0,008	-0,065	0,109	-0,025	-0,011
			0,0000	0,0014	0,0040	0,0002	0,0000
s^2		0,0056					
studentized residual			-0,14	-1,06	1,64	-0,38	-0,30
			0,14	1,06	1,64	0,38	0,30
		0	1	1	0	1	1
			5,9E-06	8,9E-06		2,0E-05	3,0E-05
			0,309	0,267		0,368	0,435
slope		6232,60713	**1,60E-04**				
intercept		0,24353					
r^2		0,8857					

		Standard Error					
		0	1	1	0	1	1
		I	II	III	IV	V	VI
[A] in [M]		4,611	3,821	3,946	3,412	3,656	3,485
[B] in [M]		3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]		0,389	1,179	1,054	1,588	1,344	1,515
KD			1,5E-05	2,9E-05		5,1E-05	6,6E-05
		4,0E-05					
SE(KD)		1,113E-05					
fb			0,565	0,405		0,283	0,234
		37,2%					
SE(fb)		7,390%					
n		4					
		1,00E-12	1,54E-05	2,93E-05	1,00E-12	5,08E-05	6,55E-05



initial r2:		0,4922				
skip outlier detection if r2 >		0,98				
		consider	outlier detection			
check, if estimation of reference assay volume becomes worse after outlier detection						
checking effect of outlier removal on intercept estimation of nt/c						
	0	1	1	1	1	1
albumin conc. [µM]		0,000	0,000	0,000	0,000	0,000
(1-fu)/fu		0,309	0,267	0,465	0,368	0,435
	5373,41661 w/o outlier		with outlier			
	0,26609	239,734	239,813	0,00 ok		
	0,3873 improvement?		yes	0,8		
	accept outlier:		**yes**			
abs(studentized residuals)		0,69	0,07	1,47	0,17	0,80	1,47
		5,92593E-06	8,88889E-06	1,33333E-05	0,00002	0,00003	
		0,3	0,3		0,4	0,4	
second tier r2:		0,3873					
skip outlier detection if r2 >		0,98					
		consider	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2							

		test article No	5	**SL 7**				
		5,000 µM					0 nm / amu	
adj. Ref 1		5,100	A		av ref ok			
Ref 2		5,600	ref diff ok		max ref ok			
Signal Reference:		5,350	5,350	5,350	5,350	5,350	5,350	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		1,900	1,100	0,800	0,800	0,780	1,100	ok

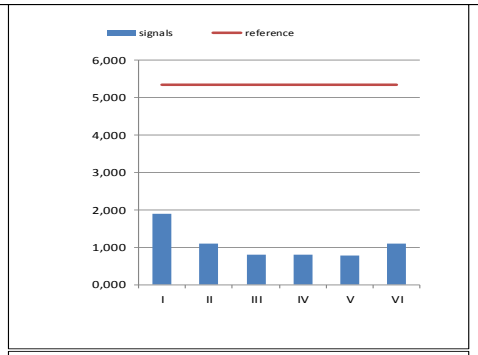
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	**inconsistent**
saturation		ok	ok	ok	ok	ok	**na**
statistics		ok	ok	ok	ok	ok	**na**

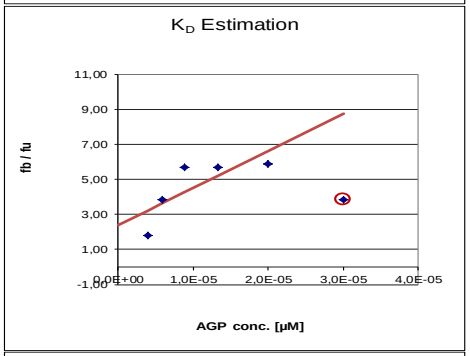
QC index: **6,4** **k_D= 1,20E-06** **fb=** **94,3% ± 1,843%** **r^2= 0,60370**

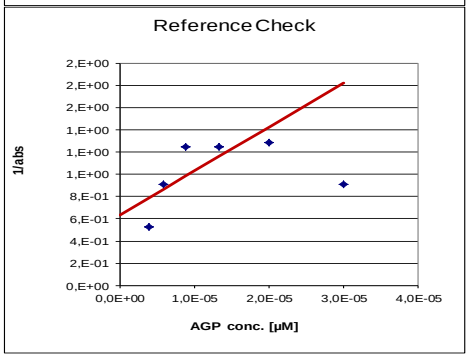
warning messages: data not consistent, poor model fit

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	1	1	0
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		5,26E-01	9,09E-01	1,25E+00	1,25E+00	1,28E+00	9,09E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		64,5%	79,4%	85,0%	85,0%	85,4%	79,4%
(1-fu)/fu		1,816	3,864	5,688	5,688	5,859	3,864
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		1,816	3,864	5,688	5,688	5,859	3,864
slope		47822,27158					
intercept		3,80848					
r^2		0,0874					
prediction: (1-fu)/fu		4,0E+00	4,1E+00	4,2E+00	4,4E+00	4,8E+00	5,2E+00
outlier due to saturation and consistency		1	1	1	1	1	0
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	
(1-fu)/fu		1,816	3,864	5,688	5,688	5,859	
slope		211866,73332					
intercept		2,37508					
r^2		0,6037					
prediction		3,212	3,631	4,258	5,200	6,612	
residuals		-1,396	0,233	1,429	0,488	-0,753	
standarized residuals		0,000	0,212	1,298	0,443	0,000	
Threshold		1,5					
statistical outlier	5	1	1	1	1	1	0
		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	
		1,816	3,864	5,688	5,688	5,859	
slope		211866,73332	**4,72E-06**				
intercept		2,37508					
r^2		0,6037					
prediction	2,375	3,212	3,631	4,258	5,200	6,612	8,731
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	3,864
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		3,22	3,97	4,25	4,25	4,27	3,97
free protein con. [µM]		0,73	1,95	4,64	9,08	15,73	26,03
free protein con. [M]	0	7,3E-07	2,0E-06	4,6E-06	9,1E-06	1,6E-05	2,6E-05
binding sites covered [%]		81,6%	67,0%	47,8%	31,9%	21,4%	13,2%

warning? invalid assumption [AP]<<[P]







reg	**4,72E-06**	**0,60370**
avg	**1,20E-06**	0
choice	**1,20E-06**	

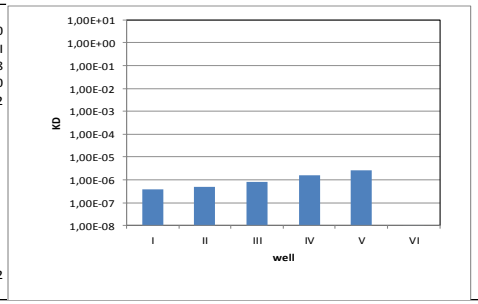
value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		5,26E-01	9,09E-01	1,25E+00	1,25E+00	1,28E+00	9,09E-01
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	
valid 1/signal		5,3E-01	9,1E-01	1,3E+00	1,3E+00	1,3E+00	
slope		39601,25856					
intercept		0,63086					
r^2		0,6037					
predicted 1/signal	1	7,9E-01	8,7E-01	9,8E-01	1,2E+00	1,4E+00	1,8E+00
Predicted Reference Value		1,59					
Actual Reference Value		5,35					
Relative deviation		70,4%					

				QC index: 6,4									warning messages
no. data points	9	reference	7	r^2 MA predict	4	ref. Treatment	10	ref > signal	10	consistency	6		data points
		10,0%		0,9999		A	10	6	10	6			reference
6		20,0%		0,999		M		5		5	6		ref. Treatment
5	9	50,0%		0,99		R		4		4			ref > signal
4		100,0%	7	0,9		slopes	10	3		3			consistency data not consisten X
3		200,0%	5	0,8		1/abs > 0	5	2		2			r^2 MA prediction poor model fit, X
2		500,0%	3	0,7		c(lipid)/c(buffer) >0	10	1		intercept			slopes
1		<500%		0,6	4			0		2,38	0		intercept poor intercept fit, X
0				0,5	3								binding invalid assumptior X
				<0.5									summary data not consistent, poor model fit
													poor intercept fit, invalid assumption [AP]<<[P]

		1. step outlier detection															
mean x		0,000		n	5												
$(x_i\text{-mean}(x))^2$		4,2E-11	2,0E-11	2,3E-12	8,5E-12	9,2E-11				initial r2:		0,6037					
$S(x_i\text{-mean}(x))^2$		1,6E-10								skip outlier detection if r2 >		0,98					
		0,55	0,68	0,79	0,75	0,24						consider	outlier detection				
residuals		-1,396	0,233	1,429	0,488	-0,753											
		1,2130								check, if estimation of reference assay volume becomes worse after outlier detection							
		0,6499	0,0181	0,6808	0,0792	0,1892											
s^2		1,6173										checking effect of outlier removal on intercept estimation of nt/c					
studentized residual		-1,49	0,22	1,27	0,44	-1,20					1	1	1	1	1	0	
		1,49	0,22	1,27	0,44	1,20				albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000		
		1	1	1	1	1	0			(1-fu)/fu	1,8	3,864	5,688	5,688	5,859		
		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05					211866,73332	w/o outlier	with outlier				
		1,816	3,864	5,688	5,688	5,859					2,37508	237,625	237,625	0,00	ok		
slope		211866,73332									0,6037	improvement?	no	1,0			
intercept		2,37508										accept outlier:	**na**				
r^2		0,6037								abs(studentized residuals)	1,49	0,22	1,27	0,44	1,20		1,49
		2. step outlier detection									3,95062E-06	5,92593E-06	8,88889E-06	1,33333E-05	0,00002		
mean x		0,000		n	5							3,9	5,7	5,7	5,9		
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00	0,00											
$S(x_i\text{-mean}(x))^2$		0,00															
		0,55	0,68	0,79	0,75	0,24											
residuals		-1,396	0,233	1,429	0,488	-0,753				second tier r2:		0,6037					
		0,6499	0,0181	0,6808	0,0792	0,1892				skip outlier detection if r2 >		0,98					
s^2		1,6173										consider	outlier detection				
studentized residual		-1,49	0,22	1,27	0,44	-1,20											
		1,49	0,22	1,27	0,44	1,20				no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
		1	1	1	1	1	0										
		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05											
		1,816	3,864	5,688	5,688	5,859											
slope		211866,73332	**4,72E-06**														
intercept		2,37508															
r^2		0,6037															

		Standard Error				
		1	1	1	1	1	0
		I	II	III	IV	V	VI
[A] in [M]		1,776	1,028	0,748	0,748	0,729	1,028
[B] in [M]		3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]		3,224	3,972	4,252	4,252	4,271	3,972
KD		4,0E-07	5,1E-07	8,2E-07	1,6E-06	2,7E-06	
		1,2E-06					
SE(KD)		4,261E-07					
fb		0,980	0,975	0,961	0,926	0,882	
		94,5%					
SE(fb)		1,843%					
n		5					
		4,00E-07	5,06E-07	8,15E-07	1,60E-06	2,68E-06	1,00E-12



test article No 6 **SL 14**

		5,000 µM				0 nm / amu	
adj. Ref 1		108,500	R		av ref too low		
Ref 2		135,200	ref diff ok		max ref too low R		
Signal Reference:		149,300	149,300	149,300	149,300	149,300	149,300	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		149,300	143,600	53,600	45,900	61,600	16,600	ok

-

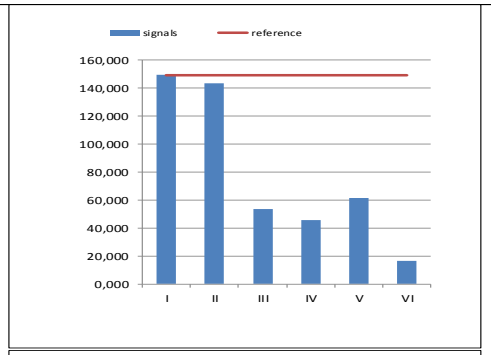
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	**inconsistent**	ok
saturation		**reference**	ok	ok	ok	**na**	ok
statistics		ok	ok	ok	ok	**na**	ok

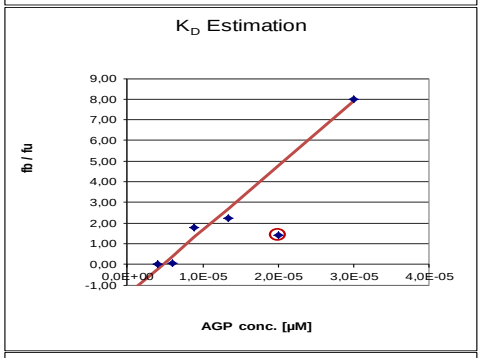
r^2 = **0,98593**

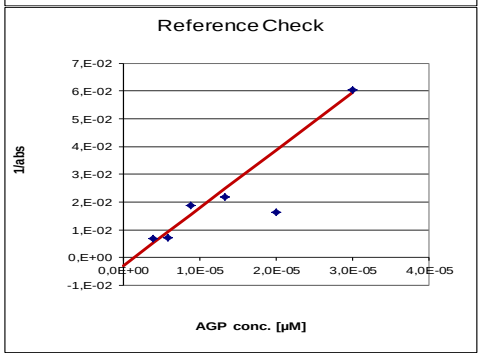
QC index: **7,3** **k_D= 3,21E-06** **fb= 86,2% ± 18,197%**

warning messages: mismatch: estimated vs measured reference, data not consistent,

		consistency check					
signal < reference	5	0	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	1	0	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		6,70E-03	6,96E-03	1,87E-02	2,18E-02	1,62E-02	6,02E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	3,8%	64,1%	69,3%	58,7%	88,9%
(1-fu)/fu		0,000	0,040	1,785	2,253	1,424	7,994
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,000	0,040	1,785	2,253	1,424	7,994
slope		268047,66044					
intercept		-1,41847					
r^2		0,7935					
prediction: (1-fu)/fu		-3,6E-01	1,7E-01	9,6E-01	2,2E+00	3,9E+00	6,6E+00
outlier due to saturation and consistency		1	1	1	1	0	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05		0,000
(1-fu)/fu		0,000	0,040	1,785	2,253		7,994
slope		311812,48501					
intercept		-1,45827					
r^2		0,9859					
prediction		-0,226	0,390	1,313	2,699		7,896
residuals		0,226	-0,350	0,472	-0,447		0,098
standarized residuals		0,582	0,000	1,213	0,000		0,252
Threshold		1,5					
statistical outlier	5	1	1	1	1	0	1
		4,0E-06	5,9E-06	8,9E-06	1,3E-05		3,0E-05
		0,000	0,040	1,785	2,253		7,994
slope		311812,48501	**3,21E-06**				
intercept		-1,45827					
r^2		0,9859					
prediction	-1,458	-0,226	0,390	1,313	2,699	4,778	7,896
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	1,424	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,19	3,20	3,46	2,94	4,44
free protein con. [µM]		3,95	5,74	5,68	9,87	17,06	25,56
free protein con. [M]	0	4,0E-06	5,7E-06	5,7E-06	9,9E-06	1,7E-05	2,6E-05
binding sites covered [%]		0,0%	3,2%	36,1%	26,0%	14,7%	14,8%
warning?							







reg	**3,21E-06**	**0,98593**	
avg	**3,88E-05**		1
choice	**3,21E-06**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		6,70E-03	6,96E-03	1,87E-02	2,18E-02	1,62E-02	6,02E-02
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06	1,3E-05		3,0E-05
valid 1/signal		6,7E-03	7,0E-03	1,9E-02	2,2E-02		6,0E-02
slope		2088,49622					
intercept		-0,00307					
r^2		0,9859					
predicted 1/signal	0	5,2E-03	9,3E-03	1,5E-02	2,5E-02	3,9E-02	6,0E-02
Predicted Reference Value		-325,79					
Actual Reference Value		149,30					
Relative deviation		318,2%					

QC index: 7,3

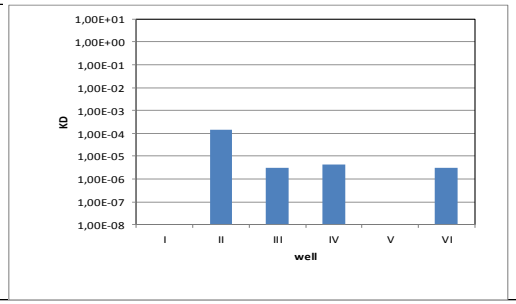
no. data points	9	reference	3	r^2 MA predict	7	ref. Treatment	10	ref > signal	8	consistency	6		warning messages	
		10,0%		0,9999		A		6		6			data points	
6		20,0%		0,999		M		5	8	5	6		reference mismatch: estimat	X
5	9	50,0%		0,99		R	10	4		4			ref. Treatment	
4		100,0%		0,9	7	slopes	10	3		3			ref > signal	
3		200,0%		0,8	6	1/abs > 0	5	2		2			consistency data not consisten	X
2		500,0%	3	0,7	5	lipid)/c(buffer) >0	10	1		intercept			r^2 MA prediction	
1		<500%		0,6	4			0		-1,46	5		slopes	
0				0,5	3								intercept	
				<0.5									binding	

summary mismatch: estimated vs measured reference, data not con

data not consistent

	1. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	7,2E-11	4,2E-11	1,2E-11	8,3E-13		3,1E-10
$S(x_i\text{-mean}(x))^2$	4,4E-10					
	0,64	0,70	0,77	0,80		0,09
residuals	0,226	-0,350	0,472	-0,447		0,098
	0,1514					
	0,0171	0,0408	0,0743	0,0665		0,0032
s^2	0,2018					
studentized residual	0,63	-0,93	1,20	-1,11		0,72
	0,63	0,93	1,20	1,11		0,72
	1	1	1	1	0	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05		3,0E-05
	0,000	0,040	1,785	2,253		7,994
slope	311812,48501					
intercept	-1,45827					
r^2	0,9859					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00		0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,64	0,70	0,77	0,80		0,09
residuals	0,226	-0,350	0,472	-0,447		0,098
	0,0171	0,0408	0,0743	0,0665		0,0032
s^2	0,2018					
studentized residual	0,63	-0,93	1,20	-1,11		0,72
	0,63	0,93	1,20	1,11		0,72
	1	1	1	1	0	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05		3,0E-05
	0,000	0,040	1,785	2,253		7,994
slope	311812,48501	**3,21E-06**				
intercept	-1,45827					
r^2	0,9859					

	Standard Error					
	0	1	1	1	0	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,809	1,795	1,537	2,063	0,556
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,000	0,191	3,205	3,463	2,937	4,444
KD		1,4E-04	3,2E-06	4,4E-06		3,2E-06
	3,9E-05					
SE(KD)	3,522E-05					
fb		0,122	0,863	0,820		0,862
	66,7%					
SE(fb)	18,197%					
n	4					
	1,00E-12	1,44E-04	3,18E-06	4,38E-06	1,00E-12	3,20E-06



initial r2:		0,9859				
skip outlier detection if r2 >		0,98				
		omit	outlier detection			
check, if estimation of reference assay volume becomes worse after outlier detection						
		checking effect of outlier removal on intercept estimation of nt/c				
		1	1	1	1	0	1
albumin conc. [µM]		0,000	0,000	0,000	0,000		0,000
(1-fu)/fu		0,0	0,040	1,785	2,253		7,994
	311812,48501	w/o outlier		with outlier			
		-1,13709	241,137	241,458	0,00	ok	
		0,9859	improvement?	yes	1,0		
			accept outlier:	**na**			
abs(studentized residuals)		0,63	0,93	1,20	1,11		0,72	1,20
		3,95062E-06	5,92593E-06	8,88889E-06	1,33333E-05		0,00003
		0,0	0,0		2,3		8,0
second tier r2:		0,9859					
skip outlier detection if r2 >		0,98					
		omit	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2							

	test article No	7	**SL 1**				
		5,000 µM				0 nm / amu	
adj. Ref 1		27,100	R		av ref too low		
Ref 2		31,400	ref diff ok		max ref too low	R	
Signal Reference:		32,000	32,000	32,000	32,000	32,000	32,000
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		32,000	25,600	24,600	27,000	24,000	23,600	ok

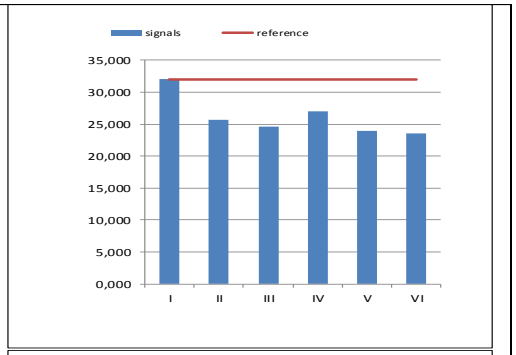
-

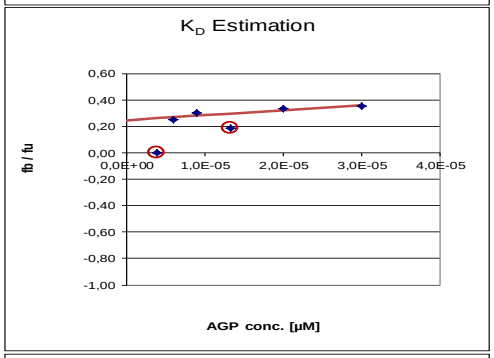
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	**inconsistent**	ok	ok
saturation	**reference**	ok	ok	**na**	ok	ok
statistics	**outlier**	ok	ok	**na**	ok	ok

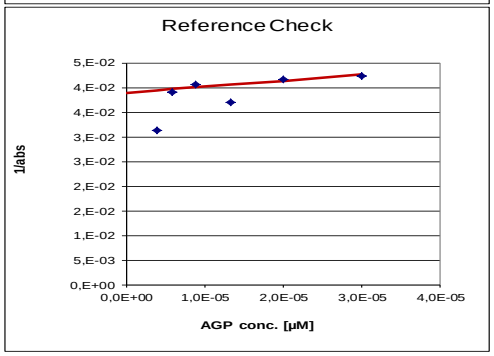
QC index: 7,9 **k_D= 2,59E-04** **fb= 7,2% ± 7,179%** **r^2= 0,85723**

warning messages: data not consistent,

		consistency check					
signal < reference	5	0	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	0	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,13E-02	3,91E-02	4,07E-02	3,70E-02	4,17E-02	4,24E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	20,0%	23,1%	15,6%	25,0%	26,3%
(1-fu)/fu		0,000	0,250	0,301	0,185	0,333	0,356
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,000	0,250	0,301	0,185	0,333	0,356
slope		9226,71262					
intercept		0,11129					
r^2		0,4764					
prediction: (1-fu)/fu		1,5E-01	1,7E-01	1,9E-01	2,3E-01	3,0E-01	3,9E-01
outlier due to saturation and consistency		1	1	1	0	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06		2,0E-05	0,000
(1-fu)/fu		0,000	0,250	0,301		0,333	0,356
slope		9184,10302					
intercept		0,12171					
r^2		0,4905					
prediction		0,158	0,176	0,203		0,305	0,397
residuals		-0,158	0,074	0,097		0,028	-0,041
standarized residuals		0,000	0,717	0,947		0,271	0,000
Threshold		1,5					
statistical outlier	4	0	1	1	0	1	1
			5,9E-06	8,9E-06		2,0E-05	3,0E-05
			0,250	0,301		0,333	0,356
slope		3864,20797	**2,59E-04**				
intercept		0,24741					
r^2		0,8572					
prediction	0,247	0,263	0,270	0,282	0,299	0,325	0,363
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,000	-5,000	-5,000	0,185	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	1,00	1,16	0,78	1,25	1,31
free protein con. [µM]		3,95	4,93	7,73	12,55	18,75	28,69
free protein con. [M]	0	4,0E-06	4,9E-06	7,7E-06	1,3E-05	1,9E-05	2,9E-05
binding sites covered [%]		0,0%	16,9%	13,0%	5,9%	6,3%	4,4%
warning?							







reg	**2,59E-04**	**0,85723**	
avg	**4,56E-05**		1
choice	**2,59E-04**		

value of "-5.0" places outlier markers below visible chart area

			reference QC				
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,13E-02	3,91E-02	4,07E-02	3,70E-02	4,17E-02	4,24E-02
valid albumin conc. [µM]			5,9E-06	8,9E-06		2,0E-05	3,0E-05
valid 1/signal			3,9E-02	4,1E-02		4,2E-02	4,2E-02
slope		120,75650					
intercept		0,03898					
r^2		0,8572					
predicted 1/signal	0	3,9E-02	4,0E-02	4,0E-02	4,1E-02	4,1E-02	4,3E-02
Predicted Reference Value		25,65					
Actual Reference Value		32,00					
Relative deviation		19,8%					

QC index: 7,9

no. data points	6	reference	9	r^2 MA prediction	6	ref. Treatment	10	ref > signal	8	consistency	6	warning messages				
		10,0%		0,9999		A		6		6		data points				
6		20,0%	9	0,999		M		5	8	5	6	reference				
5		50,0%	8	0,99		R	10	4		4		ref. Treatment				
4	6	100,0%	7	0,9		slopes	10	3		3		ref > signal				
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency data not cor	X			
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction				
1		<500%		0,6	4			0		0,25	10	slopes				
0				0,5	3							intercept				
				<0,5								binding				
												summary data not consistent,				

1. step outlier detection

mean x		0,000		n	5	
$(x_i\text{-mean}(x))^2$		9,6E-11	6,1E-11	2,4E-11	3,9E-11	2,6E-10
$S(x_i\text{-mean}(x))^2$		4,8E-10				
		0,60	0,67	0,75	0,72	0,25
residuals		-0,158	0,074	0,097	0,028	-0,041
		0,0106				
		0,0083	0,0018	0,0032	0,0003	0,0006
s^2		0,0141				
studentized residual		-1,71	0,76	0,95	0,28	-0,69
		1,71	0,76	0,95	0,28	0,69
		0	1	1	0	1	1
			5,9E-06	8,9E-06	2,0E-05	3,0E-05
			0,250	0,301	0,333	0,356
slope		3864,20797				
intercept		0,24741				
r^2		0,8572				

initial r2: 0,4905

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	0	1	1	0	1	1
albumin conc. [µM]		0,000	0,000		0,000	0,000
(1-fu)/fu		0,250	0,301		0,333	0,356
	3864,20797 w/o outlier		with outlier			
	0,23553	239,764	239,878	0,00 ok		
	0,8572 improvement?		yes	3,6		
	accept outlier:		**yes**			
abs(studentized residuals)		0,76	0,95		0,28	0,69	0,95
		5,92593E-06	8,88889E-06		0,00002	0,00003	
		0,3			0,3	0,4	

2. step outlier detection

mean x		0,000		n	4	
$(x_i\text{-mean}(x))^2$			0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$		0,00				
			0,46	0,60	0,71	0,23
residuals			-0,020	0,019	0,009	-0,007
			0,0002	0,0002	0,0000	0,0000
s^2		0,0005				
studentized residual			-1,41	1,15	0,48	-0,73
			1,41	1,15	0,48	0,73
		0	1	1	0	1	1
			5,9E-06	8,9E-06	2,0E-05	3,0E-05
			0,250	0,301	0,333	0,356
slope		3864,20797	**2,59E-04**			
intercept		0,24741				
r^2		0,8572				

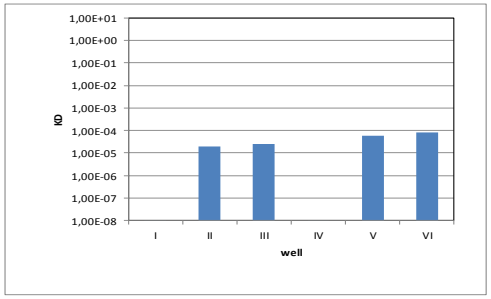
second tier r2: 0,8572

skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

		Standard Error				
	0	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,000	3,844	4,219	3,750	3,688
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,000	1,000	1,156	0,781	1,250	1,313
KD		2,0E-05	2,6E-05		5,6E-05	8,1E-05
	4,6E-05					
SE(KD)	1,416E-05					
fb		0,504	0,438		0,262	0,199
	35,1%					
SE(fb)	7,179%					
n	4					
	1,00E-12	1,97E-05	2,57E-05	1,00E-12	5,63E-05	8,06E-05



test article No 8 **SL 8**

		5,000 µM				0 nm / amu	
adj. Ref 1		28,800	A		av ref ok		
Ref 2		21,600	ref diff ok		max ref ok		
Signal Reference:		25,200	25,200	25,200	25,200	25,200	25,200
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		16,800	13,800	8,300	7,800	5,500	3,700	ok

-

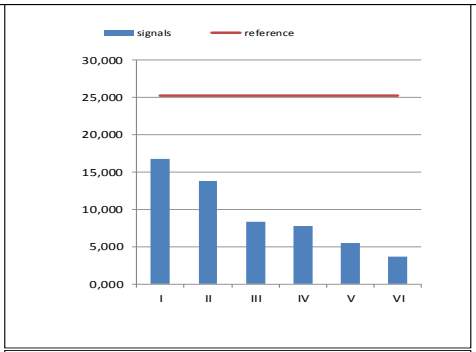
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

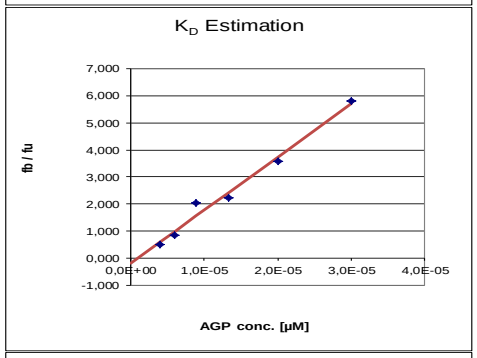
r^2= **0,98258**

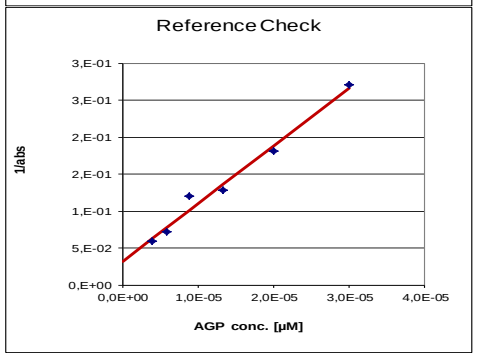
QC index: **9,4** **k_D= 5,06E-06** **fb=** **79,8% ± 1,053%**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		5,95E-02	7,25E-02	1,20E-01	1,28E-01	1,82E-01	2,70E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		33,3%	45,2%	67,1%	69,0%	78,2%	85,3%
(1-fu)/fu		0,500	0,826	2,036	2,231	3,582	5,811
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,500	0,826	2,036	2,231	3,582	5,811
slope		197509,95088					
intercept		-0,20495					
r^2		0,9826					
prediction: (1-fu)/fu		5,8E-01	9,7E-01	1,6E+00	2,4E+00	3,7E+00	5,7E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,500	0,826	2,036	2,231	3,582	5,811
slope		197509,95088					
intercept		-0,20495					
r^2		0,9826					
prediction		0,575	0,965	1,551	2,429	3,745	5,720
residuals		-0,075	-0,139	0,485	-0,198	-0,163	0,090
standarized residuals		0,000	0,000	1,876	0,000	0,000	0,350
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
		0,500	0,826	2,036	2,231	3,582	5,811
slope		197509,95088	**5,06E-06**				
intercept		-0,20495					
r^2		0,9826					
prediction	-0,205	0,575	0,965	1,551	2,429	3,745	5,720
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		1,67	2,26	3,35	3,45	3,91	4,27
free protein con. [µM]		2,28	3,66	5,54	9,88	16,09	25,73
free protein con. [M]	0	2,3E-06	3,7E-06	5,5E-06	9,9E-06	1,6E-05	2,6E-05
binding sites covered [%]		42,2%	38,2%	37,7%	25,9%	19,5%	14,2%
warning?							







reg	**5,06E-06**	**0,98258**	
avg	**4,18E-06**		1
choice	**5,06E-06**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		5,95E-02	7,25E-02	1,20E-01	1,28E-01	1,82E-01	2,70E-01
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
valid 1/signal		6,0E-02	7,2E-02	1,2E-01	1,3E-01	1,8E-01	2,7E-01
slope		7837,69646					
intercept		0,03155					
r^2		0,9826					
predicted 1/signal	0	6,3E-02	7,8E-02	1,0E-01	1,4E-01	1,9E-01	2,7E-01
Predicted Reference Value		31,70					
Actual Reference Value		25,20					
Relative deviation		20,5%					

QC index: 9,4

no. data points	10	reference	8	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%		0,9999		A	10	6	10	6	10	data points	
6	10	20,0%		0,999		M		5		5		reference	
5		50,0%	8	0,99		R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		-0,20	10	slopes	
0				0,5	3							intercept	
				<0.5								binding	
												summary	

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,075	-0,139	0,485	-0,198	-0,163	0,090
	0,0670					
	0,0014	0,0049	0,0589	0,0098	0,0067	0,0020
s^2	0,0837					
studentized residual	-0,33	-0,57	1,89	-0,75	-0,65	0,59
	0,33	0,57	1,89	0,75	0,65	0,59
	1	1	1	1	1	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
	0,500	0,826	2,036	2,231	3,582	5,811
slope	197509,95088					
intercept	-0,20495					
r^2	0,9826					
	2. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,075	-0,139	0,485	-0,198	-0,163	0,090
	0,0014	0,0049	0,0589	0,0098	0,0067	0,0020
s^2	0,0837					
studentized residual	-0,33	-0,57	1,89	-0,75	-0,65	0,59
	0,33	0,57	1,89	0,75	0,65	0,59
	1	1	1	1	1	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
	0,500	0,826	2,036	2,231	3,582	5,811
slope	197509,95088	**5,06E-06**				
intercept	-0,20495					
r^2	0,9826					

initial r2: 0,9826

skip outlier detection if r2 > 0,98

omit outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1
albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000
(1-fu)/fu	0,5	0,826	2,036	2,231	3,582	5,811
197509,95088	w/o outlier		with outlier			
	-0,11095	240,111	240,205	0,00 ok		
	0,9826	improvement?	yes	1,0		
		accept outlier:	**na**			
abs(studentized residuals)	0,33	0,57	1,89	0,75	0,65	0,59 1,89
	3,95062E-06	5,92593E-06		1,33333E-05	0,00002	0,00003
	0,5	0,8		2,2	3,6	5,8

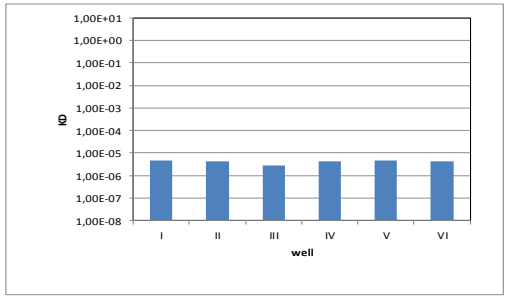
second tier r2: 0,9826

skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	3,333	2,738	1,647	1,548	1,091	0,734
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	1,667	2,262	3,353	3,452	3,909	4,266
KD	4,6E-06	4,4E-06	2,7E-06	4,4E-06	4,5E-06	4,4E-06
	4,2E-06					
SE(KD)	2,928E-07					
fb	0,814	0,818	0,880	0,819	0,817	0,819
	82,8%					
SE(fb)	1,053%					
n	6					
	4,57E-06	4,44E-06	2,72E-06	4,43E-06	4,49E-06	4,43E-06



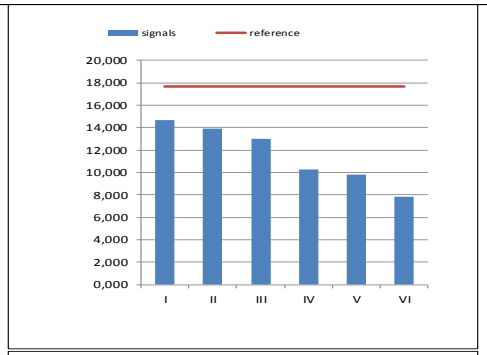
	test article No	9	**SL 12**				
	5,000 µM					0 nm / amu	
adj. Ref 1	18,600	A		av ref ok			
Ref 2	16,700	ref diff ok		max ref ok			
Signal Reference:	17,650	17,650	17,650	17,650	17,650	17,650	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	14,700	13,900	13,000	10,300	9,800	7,800	ok

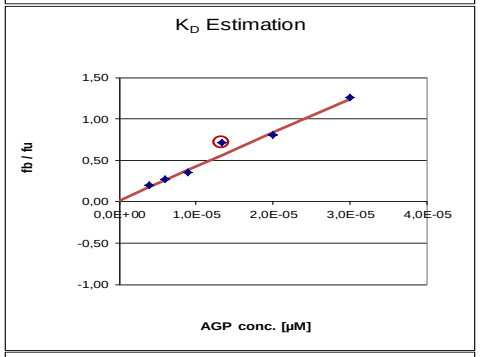
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	**outlier**	ok	ok

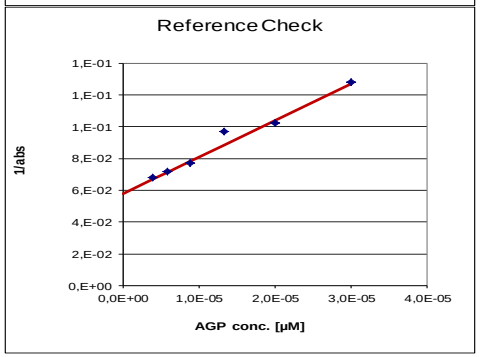
QC index: **9,6** **k_D= 2,46E-05** **fb=** **44,9% ± 1,686%** **r^2= 0,99676**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		6,80E-02	7,19E-02	7,69E-02	9,71E-02	1,02E-01	1,28E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		16,7%	21,2%	26,3%	41,6%	44,5%	55,8%
(1-fu)/fu		0,201	0,270	0,358	0,714	0,801	1,263
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,201	0,270	0,358	0,714	0,801	1,263
slope		40596,94868					
intercept		0,04544					
r^2		0,9733					
prediction: (1-fu)/fu		2,1E-01	2,9E-01	4,1E-01	5,9E-01	8,6E-01	1,3E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,201	0,270	0,358	0,714	0,801	1,263
slope		40596,94868					
intercept		0,04544					
r^2		0,9733					
prediction		0,206	0,286	0,406	0,587	0,857	1,263
residuals		-0,005	-0,016	-0,049	0,127	-0,056	-0,001
standarized residuals		0,000	0,000	0,000	1,916	0,000	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	0	1	1
		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
		0,201	0,270	0,358		0,801	1,263
slope		40706,97023	**2,46E-05**				
intercept		0,01855					
r^2		0,9968					
prediction	0,019	0,179	0,260	0,380	0,561	0,833	1,240
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	0,714	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,84	1,06	1,32	2,08	2,22	2,79
free protein con. [µM]		3,11	4,86	7,57	11,25	17,78	27,21
free protein con. [M]	0	3,1E-06	4,9E-06	7,6E-06	1,1E-05	1,8E-05	2,7E-05
binding sites covered [%]		21,2%	17,9%	14,8%	15,6%	11,1%	9,3%
warning?							







reg	**2,46E-05**	**0,99676**	
avg	**1,97E-05**		1
choice	**2,46E-05**		

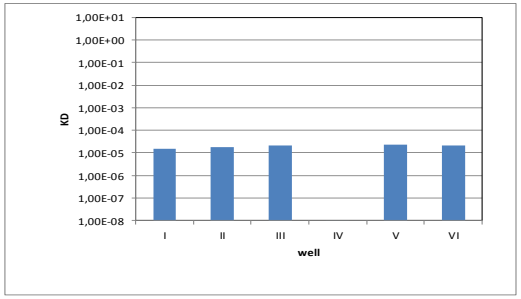
value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		6,80E-02	7,19E-02	7,69E-02	9,71E-02	1,02E-01	1,28E-01
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
valid 1/signal		6,8E-02	7,2E-02	7,7E-02		1,0E-01	1,3E-01
slope		2306,34392					
intercept		0,05771					
r^2		0,9968					
predicted 1/signal	0	6,7E-02	7,1E-02	7,8E-02	8,8E-02	1,0E-01	1,3E-01
Predicted Reference Value		17,33					
Actual Reference Value		17,65					
Relative deviation		1,8%					

				QC index: 9,6								warning messages
no. data points	9	reference	10	r^2 MA prediction	8	ref. Treatment	10	ref > signal	10	consistency	10	data points
		10,0%	10	0,9999		A	10	6	10	6	10	reference
6		20,0%	9	0,999		M		5		5		ref. Treatment
5	9	50,0%	8	0,99	8	R		4		4		ref > signal
4		100,0%	7	0,9	7	slopes	10	3		3		consistency
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		r^2 MA prediction
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		slopes
1		<500%		0,6	4			0		0,02	10	intercept
0				0,5	3							binding
				<0.5								summary

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,005	-0,016	-0,049	0,127	-0,056	-0,001
	0,0044					
	0,0000	0,0001	0,0006	0,0040	0,0008	0,0000
s^2	0,0055					
studentized residual	-0,09	-0,26	-0,74	1,88	-0,88	-0,01
	0,09	0,26	0,74	1,88	0,88	0,01
	1	1	1	0	1	1
	4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
	0,201	0,270	0,358		0,801	1,263
slope	40706,97023					
intercept	0,01855					
r^2	0,9968					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00		0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,60	0,67	0,75		0,72	0,25
residuals	0,021	0,010	-0,023		-0,032	0,023
	0,0002	0,0000	0,0002		0,0003	0,0002
s^2	0,0009					
studentized residual	0,93	0,41	-0,89		-1,27	1,55
	0,93	0,41	0,89		1,27	1,55
	1	1	1	0	1	1
	4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
	0,201	0,270	0,358		0,801	1,263
slope	40706,97023	**2,46E-05**				
intercept	0,01855					
r^2	0,9968					

	Standard Error					
	1	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,164	3,938	3,683	2,918	2,776	2,210
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,836	1,062	1,317	2,082	2,224	2,790
KD	1,6E-05	1,8E-05	2,1E-05		2,2E-05	2,2E-05
	2,0E-05					
SE(KD)	1,265E-06					
fb	0,563	0,526	0,486		0,474	0,481
	50,6%					
SE(fb)	1,686%					
n	5					
	1,55E-05	1,80E-05	2,12E-05	1,00E-12	2,22E-05	2,15E-05



initial r2:		0,9733				
skip outlier detection if r2 >		0,98				
		consider	outlier detection			
check, if estimation of reference assay volume becomes worse after outlier detection						
		checking effect of outlier removal on intercept estimation of nt/c				
	1	1	1	0	1	1
albumin conc. [µM]	0,000	0,000	0,000		0,000	0,000
(1-fu)/fu	0,2	0,270	0,358		0,801	1,263
	40706,97023 w/o outlier		with outlier			
	0,04225	239,958	239,955	0,00 ok		
	0,9968 improvement?		no	8,3		
	accept outlier:		**no**			
abs(studentized residuals)	0,09	0,26	0,74		0,88	0,01 0,88
	3,95062E-06	5,92593E-06	8,88889E-06		0,00002	0,00003
	0,2	0,3	0,4			1,3
second tier r2:		0,9968				
skip outlier detection if r2 >		0,98				
		omit	outlier detection			
no outliers will be recorded if there are only 3 or less points left at step 1 or 2						

	test article No		10		**SL 9**		
		5,000 µM				0 nm / amu	
adj. Ref 1		174,400	M		av ref ok		
Ref 2		91,500	high ref diff	M	max ref ok		
Signal Reference:		174,400	174,400	174,400	174,400	174,400	174,400
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		52,500	58,500	42,300	39,000	24,400	17,200	ok

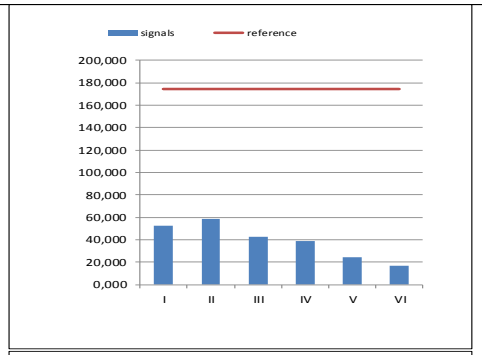
	Ref 1 <> Ref 2						
	missing values						
	1	1	1	1	1	1
	outlier detection						
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	**outlier**	ok	ok

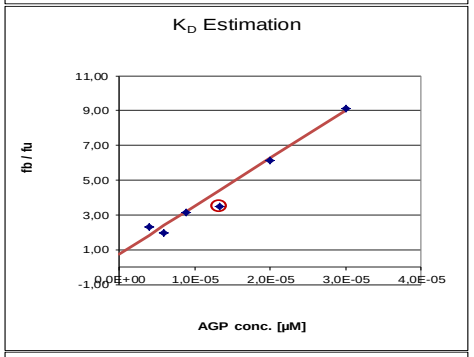
QC index: **8,4** **k_D= 3,63E-06** **fb=** **84,6% ± 2,017%** **r^2= 0,98848**

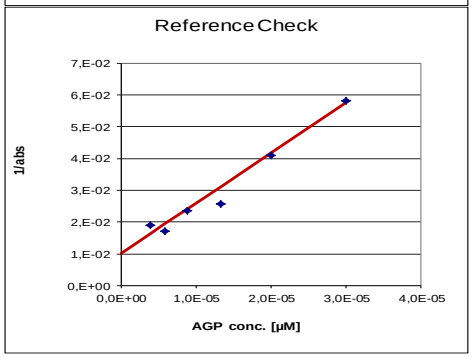
warning messages: invalid assumption [AP]<<[P]

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		1,90E-02	1,71E-02	2,36E-02	2,56E-02	4,10E-02	5,81E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		69,9%	66,5%	75,7%	77,6%	86,0%	90,1%
(1-fu)/fu		2,322	1,981	3,123	3,472	6,148	9,140
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		2,322	1,981	3,123	3,472	6,148	9,140
slope		276381,09337					
intercept		0,58239					
r^2		0,9689					
prediction: (1-fu)/fu		1,7E+00	2,2E+00	3,0E+00	4,3E+00	6,1E+00	8,9E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		2,322	1,981	3,123	3,472	6,148	9,140
slope		276381,09337					
intercept		0,58239					
r^2		0,9689					
prediction		1,674	2,220	3,039	4,267	6,110	8,874
residuals		0,648	-0,239	0,084	-0,796	0,038	0,266
standarized residuals		1,328	0,000	0,172	0,000	0,077	0,545
Threshold		1,5					
statistical outlier	5	1	1	1	0	1	1
		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
		2,322	1,981	3,123		6,148	9,140
slope		275691,03299	**3,63E-06**				
intercept		0,75102					
r^2		0,9885					
prediction	0,751	1,840	2,385	3,202	4,427	6,265	9,022
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	3,472	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		3,49	3,32	3,79	3,88	4,30	4,51
free protein con. [µM]		0,46	2,60	5,10	9,45	15,70	25,49
free protein con. [M]	0	4,6E-07	2,6E-06	5,1E-06	9,5E-06	1,6E-05	2,5E-05
binding sites covered [%]		88,5%	56,1%	42,6%	29,1%	21,5%	15,0%

warning? invalid assumption [AP]<<[P]







reg	**3,63E-06**	**0,98848**
avg	**1,70E-06**	1
choice	**3,63E-06**	

value of "-5.0" places outlier markers below visible chart area

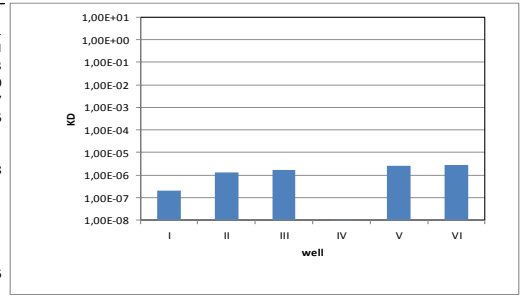
		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		1,90E-02	1,71E-02	2,36E-02	2,56E-02	4,10E-02	5,81E-02
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
valid 1/signal		1,9E-02	1,7E-02	2,4E-02		4,1E-02	5,8E-02
slope		1580,79721					
intercept		0,01004					
r^2		0,9885					
predicted 1/signal	0	1,6E-02	1,9E-02	2,4E-02	3,1E-02	4,2E-02	5,7E-02
Predicted Reference Value		99,60					
Actual Reference Value		174,40					
Relative deviation		42,9%					

QC index: 8,4

no. data points	9	reference	8	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%		0,9999		A		6	10	6	10	data points	
6		20,0%		0,999		M	10	5		5		reference	
5	9	50,0%	8	0,99		R		4		4		ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction	
1		<500%		0,6	4			0		0,75	5	slopes	
0				0,5	3							intercept	
				<0.5								binding invalid assu	X
												summary invalid assumption [AP]<<[P]	

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	0,648	-0,239	0,084	-0,796	0,038	0,266
	0,2377					
	0,1049	0,0143	0,0018	0,1583	0,0004	0,0177
s^2	0,2972					
studentized residual	1,49	-0,52	0,17	-1,60	0,08	0,92
	1,49	0,52	0,17	1,60	0,08	0,92
	1	1	1	0	1	1
	4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
	2,322	1,981	3,123		6,148	9,140
slope	275691,03299					
intercept	0,75102					
r^2	0,9885					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00		0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,60	0,67	0,75		0,72	0,25
residuals	0,482	-0,404	-0,079		-0,117	0,118
	0,0774	0,0543	0,0021		0,0046	0,0046
s^2	0,1429					
studentized residual	1,64	-1,30	-0,24		-0,37	0,62
	1,64	1,30	0,24		0,37	0,62
	1	1	1	0	1	1
	4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
	2,322	1,981	3,123		6,148	9,140
slope	275691,03299	**3,63E-06**				
intercept	0,75102					
r^2	0,9885					

	Standard Error					
	1	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	1,505	1,677	1,213	1,118	0,700	0,493
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	3,495	3,323	3,787	3,882	4,300	4,507
KD	2,0E-07	1,3E-06	1,6E-06		2,6E-06	2,8E-06
	1,7E-06					
SE(KD)	4,653E-07					
fb	0,990	0,938	0,924		0,887	0,878
	92,3%					
SE(fb)	2,017%					
n	5					
	1,96E-07	1,31E-06	1,63E-06	1,00E-12	2,55E-06	2,79E-06



initial r2:		0,9689						
skip outlier detection if r2 >		0,98						
		consider	outlier detection					
check, if estimation of reference assay volume becomes worse after outlier detection								
			checking effect of outlier removal on intercept estimation of nt/c					
			1	1	1	0	1	1
	albumin conc. [µM]		0,000	0,000	0,000		0,000	0,000
	(1-fu)/fu		2,3	1,981	3,123		6,148	9,140
		275691,03299	w/o outlier		with outlier			
		0,87206		239,128	239,418	0,00	ok	
		0,9885	improvement?		yes	2,7		
			accept outlier:		**yes**			
	abs(studentized residuals)		1,49	0,52	0,17		0,08	0,92	1,49
		3,95062E-06		5,92593E-06	8,88889E-06		0,00002	0,00003
				2,0	3,1		6,1	9,1
second tier r2:		0,9885						
skip outlier detection if r2 >		0,98						
		omit	outlier detection					
no outliers will be recorded if there are only 3 or less points left at step 1 or 2								

test article No 11 **SL 2**

		5,000 µM				0 nm / amu	
adj. Ref 1		21,000	M		av ref too low		
Ref 2		24,600	ref diff ok	M	max ref ok		
Signal Reference:		24,600	24,600	24,600	24,600	24,600	24,600
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		23,000	20,700	21,800	20,000	20,400	20,000	ok

-

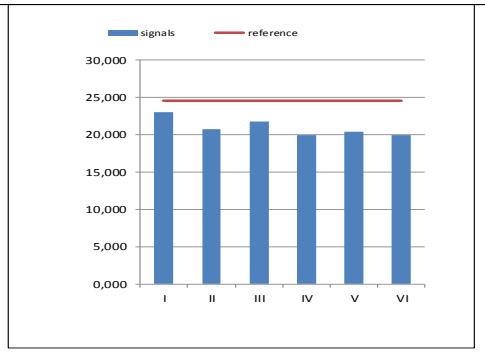
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		**outlier**	ok	**outlier**	ok	ok	ok

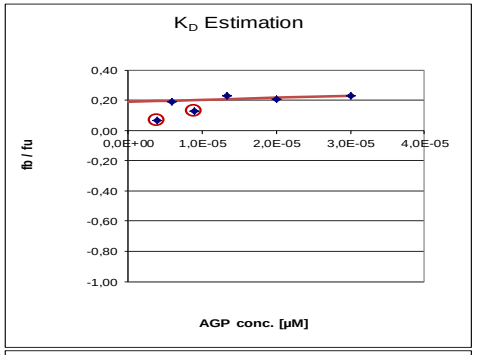
r^2= **0,43267**

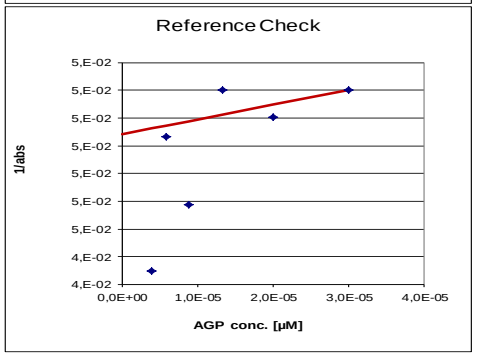
QC index: **7,8** **k_D= 7,51E-05** **fb=** **21,0% ± 6,366%**

warning messages: poor model fit,

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		4,35E-02	4,83E-02	4,59E-02	5,00E-02	4,90E-02	5,00E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		6,5%	15,9%	11,4%	18,7%	17,1%	18,7%
(1-fu)/fu		0,070	0,188	0,128	0,230	0,206	0,230
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,070	0,188	0,128	0,230	0,206	0,230
slope		4582,58421					
intercept		0,11268					
r^2		0,4968					
prediction: (1-fu)/fu		1,3E-01	1,4E-01	1,5E-01	1,7E-01	2,0E-01	2,5E-01
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,070	0,188	0,128	0,230	0,206	0,230
slope		4582,58421					
intercept		0,11268					
r^2		0,4968					
prediction		0,131	0,140	0,153	0,174	0,204	0,250
residuals		-0,061	0,049	-0,025	0,056	0,002	-0,020
standarized residuals		0,000	1,070	0,000	1,239	0,034	0,000
Threshold		1,5					
statistical outlier	4	0	1	0	1	1	1
			5,9E-06		1,3E-05	2,0E-05	3,0E-05
			0,188		0,230	0,206	0,230
slope		1303,70865	**7,67E-04**				
intercept		0,19100					
r^2		0,4327					
prediction	0,191	0,196	0,199	0,203	0,208	0,217	0,230
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,070	-5,000	0,128	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,33	0,79	0,57	0,93	0,85	0,93
free protein con. [µM]		3,63	5,13	8,32	12,40	19,15	29,07
free protein con. [M]	0	3,6E-06	5,1E-06	8,3E-06	1,2E-05	1,9E-05	2,9E-05
binding sites covered [%]		8,2%	13,4%	6,4%	7,0%	4,3%	3,1%
warning?							







reg	**7,67E-04**	**0,43267**	
avg	**7,51E-05**		0
choice	**7,51E-05**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		4,35E-02	4,83E-02	4,59E-02	5,00E-02	4,90E-02	5,00E-02
valid albumin conc. [µM]			5,9E-06		1,3E-05	2,0E-05	3,0E-05
valid 1/signal			4,8E-02		5,0E-02	4,9E-02	5,0E-02
slope		52,99629					
intercept		0,04841					
r^2		0,4327					
predicted 1/signal	0	4,9E-02	4,9E-02	4,9E-02	4,9E-02	4,9E-02	5,0E-02
Predicted Reference Value		20,65					
Actual Reference Value		24,60					
Relative deviation		16,0%					

QC index: 7,8

no. data points	6	reference	9	r^2 MA prediction	0	ref. Treatment	10	ref > signal	10	consistency	10	warning messages	
		10,0%		0,9999		A		6	10	6	10	data points	
6		20,0%	9	0,999		M	10	5		5		reference	
5		50,0%	8	0,99		R		4		4		ref. Treatment	
4	6	100,0%	7	0,9		slopes	10	3		3		ref > signal	
3		200,0%	5	0,8		1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7		c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction poor model fit,	X
1		<500%		0,6				0		0,19	10	slopes	
0				0,5								intercept	
				<0.5	0							binding	
												summary poor model fit,	

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,061	0,049	-0,025	0,056	0,002	-0,020
	0,0021					
	0,0009	0,0006	0,0002	0,0008	0,0000	0,0001
s^2	0,0026					
studentized residual	-1,51	1,14	-0,56	1,21	0,04	-0,75
	1,51	1,14	0,56	1,21	0,04	0,75
	0	1	1	1	1	1
		5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
		0,188	0,128	0,230	0,206	0,230
slope	2652,77919					
intercept	0,15508					
r^2	0,3709					

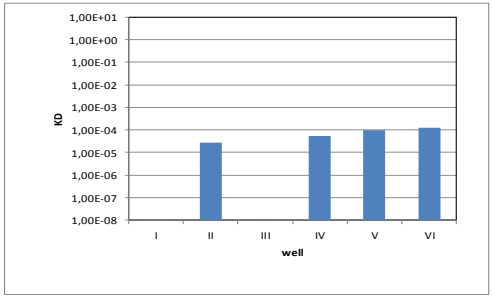
2. step outlier detection

mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
		0,55	0,68	0,79	0,75	0,24
residuals		0,018	-0,050	0,040	-0,002	-0,005
		0,0001	0,0008	0,0005	0,0000	0,0000
s^2	0,0015					
studentized residual		0,62	-1,59	1,16	-0,07	-0,25
		0,62	1,59	1,16	0,07	0,25
	0	1	0	1	1	1
		5,9E-06		1,3E-05	2,0E-05	3,0E-05
		0,188		0,230	0,206	0,230
slope	1303,70865	**7,67E-04**				
intercept	0,19100					
r^2	0,4327					

initial r2:	0,4968					
skip outlier detection if r2 >	0,98					
	consider	outlier detection				
check, if estimation of reference assay volume becomes worse after outlier detection						
	checking effect of outlier removal on intercept estimation of nt/c					
	0	1	1	1	1	1
albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000	
(1-fu)/fu	0,188	0,128	0,230	0,206	0,230	
2652,77919	w/o outlier	with outlier				
0,14727	239,853	239,887	0,00	ok		
0,3709	improvement?	yes	0,8			
	accept outlier:	**yes**				
abs(studentized residuals)	1,14	0,56	1,21	0,04	0,75	1,21
	5,92593E-06	8,88889E-06	1,33333E-05	0,00002	0,00003	
	0,2	0,1		0,2	0,2	
second tier r2:	0,3709					
skip outlier detection if r2 >	0,98					
	consider	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2						

Standard Error

	0	1	0	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,675	4,207	4,431	4,065	4,146	4,065
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,325	0,793	0,569	0,935	0,854	0,935
KD		2,7E-05		5,4E-05	9,3E-05	1,3E-04
	7,5E-05					
SE(KD)	2,177E-05					
fb		0,423		0,271	0,177	0,137
	25,2%					
SE(fb)	6,366%					
n	4					
	1,00E-12	2,72E-05	1,00E-12	5,39E-05	9,30E-05	1,26E-04



test article No 12 **SL 3**

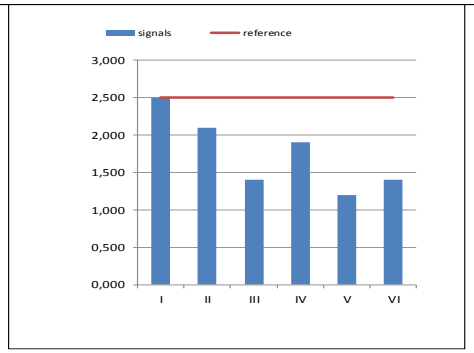
		5,000 µM					0 nm / amu
adj. Ref 1		1,800	R		av ref too low		
Ref 2		1,900	ref diff ok		max ref too low R		
Signal Reference:		2,500	2,500	2,500	2,500	2,500	2,500
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		2,500	2,100	1,400	1,900	1,200	1,400 ok

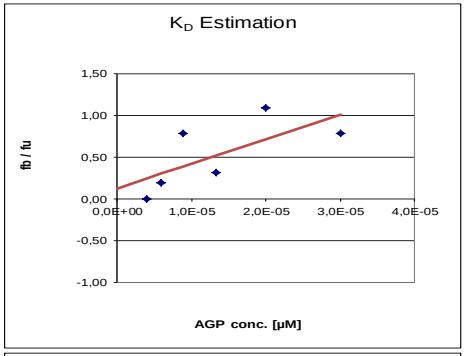
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	**reference**	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

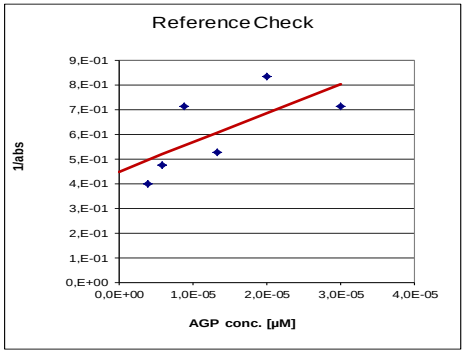
QC index: 8,1 **k_D= 2,51E-05** **fb= 44,4% ± 6,728%** **r^2= 0,48113**

warning messages: poor model fit,

		consistency check					
signal < reference	5	0	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		4,00E-01	4,76E-01	7,14E-01	5,26E-01	8,33E-01	7,14E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	16,0%	44,0%	24,0%	52,0%	44,0%
(1-fu)/fu		0,000	0,190	0,786	0,316	1,083	0,786
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,000	0,190	0,786	0,316	1,083	0,786
slope		29554,61043					
intercept		0,12244					
r^2		0,4811					
prediction: (1-fu)/fu		2,4E-01	3,0E-01	3,9E-01	5,2E-01	7,1E-01	1,0E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,000	0,190	0,786	0,316	1,083	0,786
slope		29554,61043					
intercept		0,12244					
r^2		0,4811					
prediction		0,239	0,298	0,385	0,516	0,714	1,009
residuals		-0,239	-0,107	0,401	-0,201	0,370	-0,223
standarized residuals		0,000	0,000	1,326	0,000	1,224	0,000
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
		0,000	0,190	0,786	0,316	1,083	0,786
slope		29554,61043	**3,38E-05**				
intercept		0,12244					
r^2		0,4811					
prediction	0,122	0,239	0,298	0,385	0,516	0,714	1,009
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,80	2,20	1,20	2,60	2,20
free protein con. [µM]		3,95	5,13	6,69	12,13	17,40	27,80
free protein con. [M]	0	4,0E-06	5,1E-06	6,7E-06	1,2E-05	1,7E-05	2,8E-05
binding sites covered [%]		0,0%	13,5%	24,8%	9,0%	13,0%	7,3%
warning?							







reg	**3,38E-05**	**0,48113**	
avg	**2,51E-05**		0
choice	**2,51E-05**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		4,00E-01	4,76E-01	7,14E-01	5,26E-01	8,33E-01	7,14E-01
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
valid 1/signal		4,0E-01	4,8E-01	7,1E-01	5,3E-01	8,3E-01	7,1E-01
slope		11821,84417					
intercept		0,44898					
r^2		0,4811					
predicted 1/signal	0	5,0E-01	5,2E-01	5,5E-01	6,1E-01	6,9E-01	8,0E-01
Predicted Reference Value		2,23					
Actual Reference Value		2,50					
Relative deviation		10,9%					

QC index: 8,1

no. data points	10	reference	9	r^2 MA prediction	0	ref. Treatment	10	ref > signal	8	consistency	10	warning messages	
		10,0%		0,9999		A		6		6	10	data points	
6	10	20,0%	9	0,999		M		5	8	5		reference	
5		50,0%	8	0,99		R	10	4		4		ref. Treatment	
4		100,0%	7	0,9		slopes	10	3		3		ref > signal	
3		200,0%	5	0,8		1/abs > 0	5	2		2		consistency	
2		500,0%	3	0,7		c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction poor model fit,	X
1		<500%		0,6				0		0,12	10	slopes	
0				0,5								intercept	
				<0.5	0							binding	
												summary poor model fit,	

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,239	-0,107	0,401	-0,201	0,370	-0,223
	0,0912					
	0,0143	0,0029	0,0401	0,0101	0,0342	0,0125
s^2	0,1140					
studentized residual	-0,89	-0,38	1,34	-0,65	1,26	-1,24
	0,89	0,38	1,34	0,65	1,26	1,24
	1	1	1	1	1	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
	0,000	0,190	0,786	0,316	1,083	0,786
slope	29554,61043					
intercept	0,12244					
r^2	0,4811					

initial r2: 0,4811

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1
albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000
(1-fu)/fu	0,0	0,190	0,786	0,316	1,083	0,786
	29554,61043 w/o outlier		with outlier			
	-0,10965	240,110	239,878	0,00 ok		
	0,4811 improvement?		no	1,0		
	accept outlier:		**na**			
abs(studentized residuals)	0,89	0,38	1,34	0,65	1,26	1,24	1,34
	3,95062E-06	5,92593E-06	8,88889E-06	1,33333E-05	0,00002	0,00003	
	0,0	0,2		0,3	1,1	0,8	

2. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,239	-0,107	0,401	-0,201	0,370	-0,223
	0,0143	0,0029	0,0401	0,0101	0,0342	0,0125
s^2	0,1140					
studentized residual	-0,89	-0,38	1,34	-0,65	1,26	-1,24
	0,89	0,38	1,34	0,65	1,26	1,24
	1	1	1	1	1	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
	0,000	0,190	0,786	0,316	1,083	0,786
slope	29554,61043	**3,38E-05**				
intercept	0,12244					
r^2	0,4811					

second tier r2: 0,4811

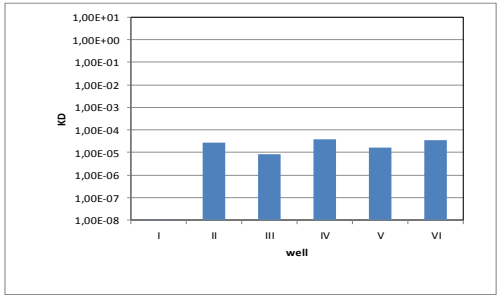
skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	0	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,200	2,800	3,800	2,400	2,800
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,000	0,800	2,200	1,200	2,600	2,200
KD		2,7E-05	8,5E-06	3,8E-05	1,6E-05	3,5E-05
	2,5E-05					
SE(KD)	5,671E-06					
fb		0,426	0,701	0,342	0,555	0,361
	47,7%					
SE(fb)	6,728%					
n	5					
	1,00E-12	2,69E-05	8,51E-06	3,84E-05	1,61E-05	3,54E-05



test article No 13 **SL 13**

	5,000 µM					0 nm / amu	
adj. Ref 1	40,700	A		av ref ok			
Ref 2	43,800	ref diff ok		max ref ok			
Signal Reference:	42,250	42,250	42,250	42,250	42,250	42,250	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	26,700	26,200	23,700	20,200	18,200	18,200	ok

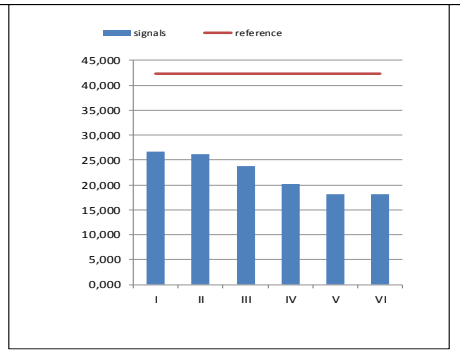
	-					
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

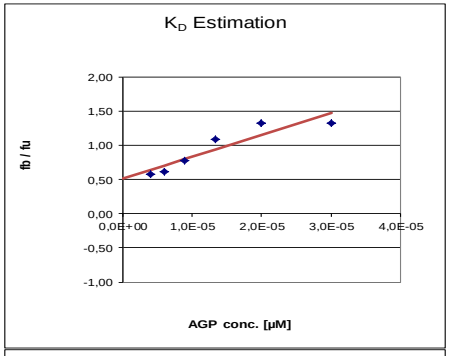
r^2= **0,84998**

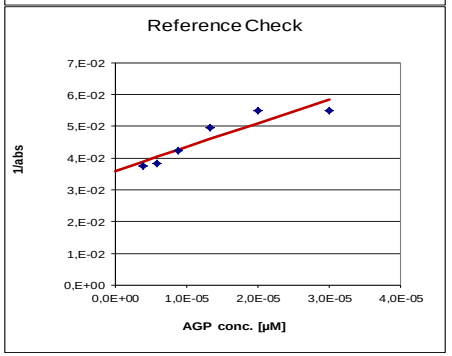
QC index: **8,4** **k_D= 3,15E-05** **fb=** **38,8% ± 4,961%**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,75E-02	3,82E-02	4,22E-02	4,95E-02	5,49E-02	5,49E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		36,8%	38,0%	43,9%	52,2%	56,9%	56,9%
(1-fu)/fu		0,582	0,613	0,783	1,092	1,321	1,321
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,582	0,613	0,783	1,092	1,321	1,321
slope		31714,35210					
intercept		0,51807					
r^2		0,8500					
prediction: (1-fu)/fu		6,4E-01	7,1E-01	8,0E-01	9,4E-01	1,2E+00	1,5E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,582	0,613	0,783	1,092	1,321	1,321
slope		31714,35210					
intercept		0,51807					
r^2		0,8500					
prediction		0,643	0,706	0,800	0,941	1,152	1,470
residuals		-0,061	-0,093	-0,017	0,151	0,169	-0,148
standarized residuals		0,000	0,000	0,000	1,149	1,290	0,000
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
		0,582	0,613	0,783	1,092	1,321	1,321
slope		31714,35210	**3,15E-05**				
intercept		0,51807					
r^2		0,8500					
prediction	0,518	0,643	0,706	0,800	0,941	1,152	1,470
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		1,84	1,90	2,20	2,61	2,85	2,85
free protein con. [µM]		2,11	4,03	6,69	10,72	17,15	27,15
free protein con. [M]	0	2,1E-06	4,0E-06	6,7E-06	1,1E-05	1,7E-05	2,7E-05
binding sites covered [%]		46,6%	32,1%	24,7%	19,6%	14,2%	9,5%
warning?							







reg	**3,15E-05**	**0,84998**	
avg	**1,04E-05**		1
choice	**3,15E-05**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		3,75E-02	3,82E-02	4,22E-02	4,95E-02	5,49E-02	5,49E-02
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
valid 1/signal		3,7E-02	3,8E-02	4,2E-02	5,0E-02	5,5E-02	5,5E-02
slope		750,63555					
intercept		0,03593					
r^2		0,8500					
predicted 1/signal	0	3,9E-02	4,0E-02	4,3E-02	4,6E-02	5,1E-02	5,8E-02
Predicted Reference Value		27,83					
Actual Reference Value		42,25					
Relative deviation		34,1%					

QC index: 8,4

no. data points	10	reference	8	r^2 MA prediction	6	ref. Treatment	10	ref > signal	10	consistency	10		warning messages
		10,0%		0,9999		A	10	6	10	6	10		data points
6	10	20,0%		0,999		M		5		5			reference
5		50,0%	8	0,99		R		4		4			ref. Treatment
4		100,0%	7	0,9		slopes	10	3		3			ref > signal
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			consistency
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept			r^2 MA prediction
1		<500%		0,6	4			0		0,52	5		slopes
0				0,5	3								intercept
				<0.5									binding
													summary

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,061	-0,093	-0,017	0,151	0,169	-0,148
	0,0172					
	0,0009	0,0022	0,0001	0,0057	0,0071	0,0055
s^2	0,0215					
studentized residual	-0,52	-0,76	-0,13	1,13	1,33	-1,90
	0,52	0,76	0,13	1,13	1,33	1,90
	1	1	1	1	1	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
	0,582	0,613	0,783	1,092	1,321	1,321
slope	31714,35210					
intercept	0,51807					
r^2	0,8500					

2. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	-0,061	-0,093	-0,017	0,151	0,169	-0,148
	0,0009	0,0022	0,0001	0,0057	0,0071	0,0055
s^2	0,0215					
studentized residual	-0,52	-0,76	-0,13	1,13	1,33	-1,90
	0,52	0,76	0,13	1,13	1,33	1,90
	1	1	1	1	1	1
	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
	0,582	0,613	0,783	1,092	1,321	1,321
slope	31714,35210	**3,15E-05**				
intercept	0,51807					
r^2	0,8500					

initial r2: 0,8500

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1	
albumin conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000	
(1-fu)/fu	0,6	0,613	0,783	1,092	1,321	1,321	
	31714,35210 w/o outlier		with outlier				
	0,36421	239,636	239,482	0,00 ok			
	0,8500 improvement?		no	1,0			
	accept outlier:		**na**				
abs(studentized residuals)	0,52	0,76	0,13	1,13	1,33	1,90	1,33
	3,95062E-06	5,92593E-06	8,88889E-06	1,33333E-05	0,00002	0,00003	
	0,6	0,6	0,8	1,1		1,3	

second tier r2: 0,8500

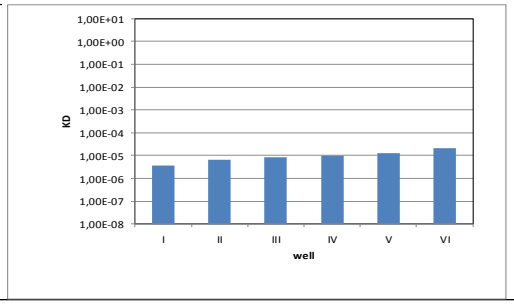
skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	3,160	3,101	2,805	2,391	2,154	2,154
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	1,840	1,899	2,195	2,609	2,846	2,846
KD	3,6E-06	6,6E-06	8,6E-06	9,8E-06	1,3E-05	2,1E-05
	1,0E-05					
SE(KD)	2,408E-06					
fb	0,847	0,753	0,700	0,671	0,606	0,493
	67,8%					
SE(fb)	4,961%					
n	6					
	3,62E-06	6,57E-06	8,55E-06	9,82E-06	1,30E-05	2,05E-05



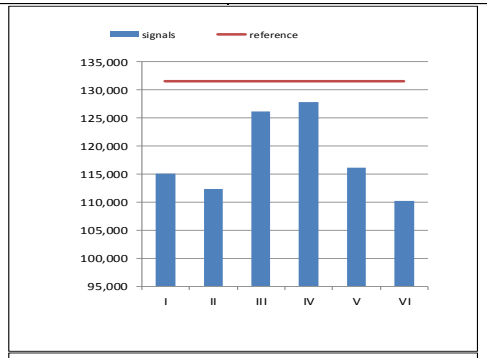
test article No 14 **SL 11**

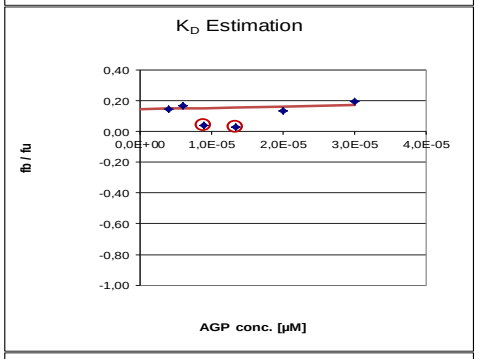
		5,000 µM				0 nm / amu	
adj. Ref 1		131,600	A		av ref ok		
Ref 2		131,500	ref diff ok		max ref ok		
Signal Reference:		131,550	131,550	131,550	131,550	131,550	131,550
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		115,000	112,400	126,200	127,800	116,100	110,200	ok
				missing values			
		1	1	1	1	1	1
				outlier detection			
consistency		ok	ok	**inconsistent**	**inconsistent**	ok	ok
saturation		ok	ok	**na**	**na**	ok	ok
statistics		ok	ok	**na**	**na**	ok	ok

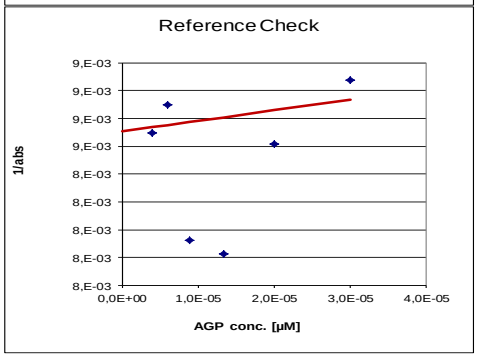
QC index: **6,7** **k_D= 8,75E-05** **fb=** **18,6% ± 9,092%** **r^2= 0,20445**

warning messages: data not consistent, poor model fit,

			consistency check				
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	4	1	1	0	0	1	1
			removal of outliers due to saturation				
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		8,70E-03	8,90E-03	7,92E-03	7,82E-03	8,61E-03	9,07E-03
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		12,6%	14,6%	4,1%	2,9%	11,7%	16,2%
(1-fu)/fu		0,144	0,170	0,042	0,029	0,133	0,194
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,144	0,170	0,042	0,029	0,133	0,194
slope		2201,94239					
intercept		0,08868					
r^2		0,1023					
prediction: (1-fu)/fu		9,7E-02	1,0E-01	1,1E-01	1,2E-01	1,3E-01	1,5E-01
outlier due to saturation and consistency		1	1	0	0	1	1
			statistical outlier detection & removal				
albumin conc. [M]		4,0E-06	5,9E-06			2,0E-05	0,000
(1-fu)/fu		0,144	0,170			0,133	0,194
slope		1001,50554					
intercept		0,14528					
r^2		0,2045					
prediction		0,149	0,151			0,165	0,175
residuals		-0,005	0,019			-0,032	0,018
standarized residuals		0,000	0,788			0,000	0,757
Threshold		1,5					
statistical outlier	4	1	1	0	0	1	1
		4,0E-06	5,9E-06			2,0E-05	3,0E-05
		0,144	0,170			0,133	0,194
slope		1001,50554	**9,98E-04**				
intercept		0,14528					
r^2		0,2045					
prediction	0,145	0,149	0,151	0,154	0,159	0,165	0,175
			outlier				
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	0,042	0,029	-5,000	-5,000
			Binding QC				
no. of binding sites		1					
conc. protein-compound complex [µM]		0,63	0,73	0,20	0,14	0,59	0,81
free protein con. [µM]		3,32	5,20	8,69	13,19	19,41	29,19
free protein con. [M]	0	3,3E-06	5,2E-06	8,7E-06	1,3E-05	1,9E-05	2,9E-05
binding sites covered [%]		15,9%	12,3%	2,3%	1,1%	2,9%	2,7%
warning?							







reg	**9,98E-04**	**0,20445**	
avg	**8,75E-05**		0
choice	**8,75E-05**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		8,70E-03	8,90E-03	7,92E-03	7,82E-03	8,61E-03	9,07E-03
valid albumin conc. [µM]		4,0E-06	5,9E-06			2,0E-05	3,0E-05
valid 1/signal		8,7E-03	8,9E-03			8,6E-03	9,1E-03
slope		7,61312					
intercept		0,00871					
r^2		0,2045					
predicted 1/signal	0	8,7E-03	8,8E-03	8,8E-03	8,8E-03	8,9E-03	8,9E-03
Predicted Reference Value		114,86					
Actual Reference Value		131,55					
Relative deviation		12,7%					

QC index: 6,7

no. data points	6	reference	9	r^2 MA prediction	0	ref. Treatment	10	ref > signal	10	consistency	3	warning messages		
		10,0%		0,9999		A	10	6	10	6		data points		
6		20,0%	9	0,999		M		5		5		reference		
5		50,0%	8	0,99		R		4		4	3	ref. Treatment		
4	6	100,0%	7	0,9		slopes	10	3		3		ref > signal		
3		200,0%	5	0,8		1/abs > 0	5	2		2		consistency data not consi	X	
2		500,0%	3	0,7		c(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction poor model fit	X	
1		<500%		0,6				0		0,15	10	slopes		
0				0,5								intercept		
				<0.5	0							binding		
												summary data not consistent, poor model fit,		

1. step outlier detection

mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	1,2E-10	8,2E-11			2,5E-11	2,3E-10
$S(x_i\text{-mean}(x))^2$	4,5E-10					
	0,48	0,57			0,69	0,25
residuals	-0,005	0,019			-0,032	0,018
	0,0006					
	0,0000	0,0002			0,0005	0,0002
s^2	0,0009					
studentized residual	-0,26	0,85			-1,30	1,23
	0,26	0,85			1,30	1,23
	1	1	0	0	1	1
	4,0E-06	5,9E-06			2,0E-05	3,0E-05
	0,144	0,170			0,133	0,194
slope	1001,50554					
intercept	0,14528					
r^2	0,2045					

initial r2: 0,2045
skip outlier detection if r2 > 0,98
consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	0	0	1	1
albumin conc. [µM]	0,000	0,000			0,000	0,000
(1-fu)/fu	0,1	0,170			0,133	0,194
	1001,50554 w/o outlier		with outlier			
	0,16313	239,837	239,855	0,00 ok		
	0,2045 improvement?		yes	1,0		
	accept outlier:		**na**			
abs(studentized residuals)	0,26	0,85			1,30	1,23	1,30
	3,95062E-06	5,92593E-06			0,00002	0,00003	
	0,1	0,2				0,2	

2. step outlier detection

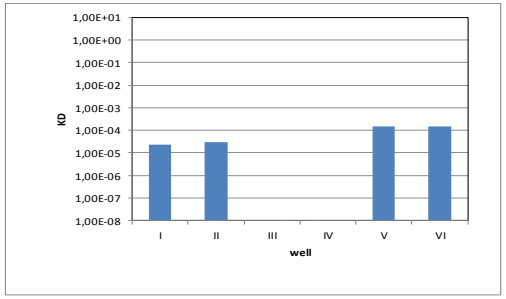
mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	0,00	0,00			0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,48	0,57			0,69	0,25
residuals	-0,005	0,019			-0,032	0,018
	0,0000	0,0002			0,0005	0,0002
s^2	0,0009					
studentized residual	-0,26	0,85			-1,30	1,23
	0,26	0,85			1,30	1,23
	1	1	0	0	1	1
	4,0E-06	5,9E-06			2,0E-05	3,0E-05
	0,144	0,170			0,133	0,194
slope	1001,50554	**9,98E-04**				
intercept	0,14528					
r^2	0,2045					

second tier r2: 0,2045
skip outlier detection if r2 > 0,98
consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	0	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,371	4,272	4,797	4,857	4,413	4,189
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,629	0,728	0,203	0,143	0,587	0,811
KD	2,3E-05	3,1E-05			1,5E-04	1,5E-04
	8,8E-05					
SE(KD)	3,511E-05					
fb	0,464	0,396			0,121	0,117
	27,4%					
SE(fb)	9,092%					
n	4					
	2,31E-05	3,05E-05	1,00E-12	1,00E-12	1,46E-04	1,51E-04



test article No 15 **SL 10**

		5,000 µM				0 nm / amu	
adj. Ref 1		9,000	M		av ref too low		
Ref 2		5,300	high ref diff	M	max ref ok		
Signal Reference:		9,000	9,000	9,000	9,000	9,000	9,000
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		8,700	7,400	6,200	5,700	1,700	1,300	ok

Ref 1 <> Ref 2

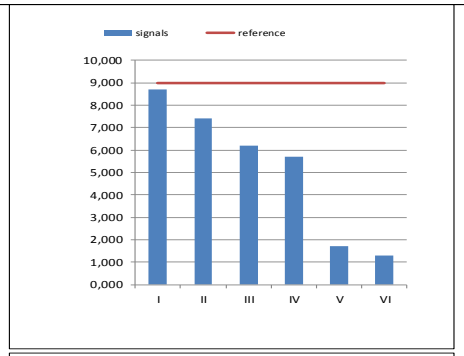
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		ok	ok	ok	**outlier**	ok	ok

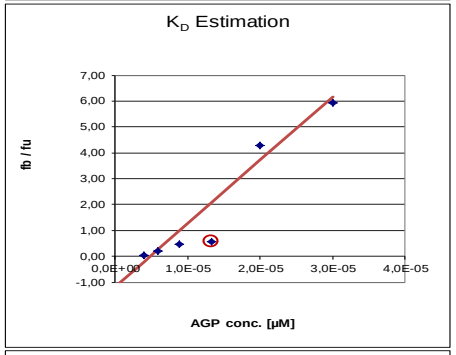
r^2= **0,97486**

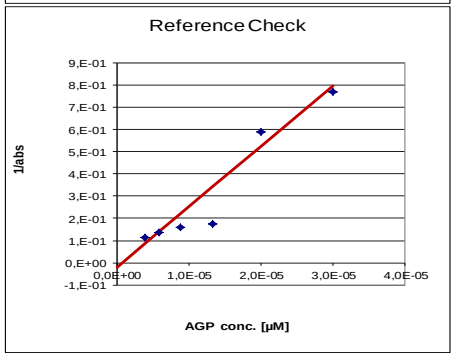
QC index: **8,0** **k_D= 4,07E-06** **fb=** **83,1% ± 12,707%**

warning messages: mismatch: estimated vs measured reference,

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		1,15E-01	1,35E-01	1,61E-01	1,75E-01	5,88E-01	7,69E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		3,3%	17,8%	31,1%	36,7%	81,1%	85,6%
(1-fu)/fu		0,034	0,216	0,452	0,579	4,294	5,923
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		0,034	0,216	0,452	0,579	4,294	5,923
slope		246601,11032					
intercept		-1,45787					
r^2		0,9179					
prediction: (1-fu)/fu		-4,8E-01	3,5E-03	7,3E-01	1,8E+00	3,5E+00	5,9E+00
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	0,000
(1-fu)/fu		0,034	0,216	0,452	0,579	4,294	5,923
slope		246601,11032					
intercept		-1,45787					
r^2		0,9179					
prediction		-0,484	0,003	0,734	1,830	3,474	5,940
residuals		0,518	0,213	-0,283	-1,251	0,820	-0,017
standarized residuals		0,714	0,293	0,000	0,000	1,130	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	0	1	1
		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
		0,034	0,216	0,452		4,294	5,923
slope		245515,99350	**4,07E-06**				
intercept		-1,19270					
r^2		0,9749					
prediction	-1,193	-0,223	0,262	0,990	2,081	3,718	6,173
		outlier					
effective albumin conc. [M]		4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
(1-fu)/fu		-5,000	-5,000	-5,000	0,579	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,17	0,89	1,56	1,83	4,06	4,28
free protein con. [µM]		3,78	5,04	7,33	11,50	15,94	25,72
free protein con. [M]	0	3,8E-06	5,0E-06	7,3E-06	1,2E-05	1,6E-05	2,6E-05
binding sites covered [%]		4,2%	15,0%	17,5%	13,8%	20,3%	14,3%
warning?							







reg	**4,07E-06**	**0,97486**	
avg	**3,15E-05**		1
choice	**4,07E-06**		

value of "-5.0" places outlier markers below visible chart area

reference QC

albumin conc. [M]	0	4,0E-06	5,9E-06	8,9E-06	1,3E-05	2,0E-05	3,0E-05
1/signal in TRANSIL well		1,15E-01	1,35E-01	1,61E-01	1,75E-01	5,88E-01	7,69E-01
valid albumin conc. [µM]		4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
valid 1/signal		1,1E-01	1,4E-01	1,6E-01		5,9E-01	7,7E-01
slope		27279,55483					
intercept		-0,02141					
r^2		0,9749					
predicted 1/signal	0	8,6E-02	1,4E-01	2,2E-01	3,4E-01	5,2E-01	8,0E-01
Predicted Reference Value		-46,70					
Actual Reference Value		9,00					
Relative deviation		618,9%					

QC index: 8,0

no. data points	9	reference	0	r^2 MA prediction	7	ref. Treatment	10	ref > signal	10	consistency	10
		10,0%		0,9999		A		6	10	6	10
6		20,0%		0,999		M	10	5		5	
5	9	50,0%		0,99		R		4		4	
4		100,0%		0,9	7	slopes	10	3		3	
3		200,0%		0,8	6	1/abs > 0	5	2		2	
2		500,0%		0,7	5	c(lipid)/c(buffer) >0	10	1		intercept	
1		<500%	0	0,6	4			0		-1,19	5
0				0,5	3						
				<0.5							

warning messages

data points	
reference mismatch: e	X
ref. Treatment	
ref > signal	
consistency	
r^2 MA prediction	
slopes	
intercept	
binding	

summary mismatch: estimated vs measured reference,

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	9,5E-11	6,0E-11	2,3E-11	1,2E-13	4,0E-11	2,7E-10
$S(x_i\text{-mean}(x))^2$	4,8E-10					
	0,64	0,71	0,79	0,83	0,75	0,28
residuals	0,518	0,213	-0,283	-1,251	0,820	-0,017
	0,5263					
	0,0671	0,0113	0,0200	0,3914	0,1681	0,0001
s^2	0,6579					
studentized residual	0,80	0,31	-0,39	-1,69	1,17	-0,04
	0,80	0,31	0,39	1,69	1,17	0,04
	1	1	1	0	1	1
	4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
	0,034	0,216	0,452		4,294	5,923
slope	245515,99350					
intercept	-1,19270					
r^2	0,9749					

initial r2: 0,9179

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	0	1	1
albumin conc. [µM]	0,000	0,000	0,000		0,000	0,000
(1-fu)/fu	0,0	0,216	0,452		4,294	5,923
	245515,99350	w/o outlier	with outlier			
	-1,44931	241,449	241,458	0,00	ok	
	0,9749	improvement?	yes	3,3		
		accept outlier:	**yes**			
abs(studentized residuals)	0,80	0,31	0,39		1,17	0,04	1,17
	3,95062E-06	5,92593E-06	8,88889E-06		0,00002	0,00003	
	0,0	0,2	0,5			5,9	

2. step outlier detection

mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00		0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,60	0,67	0,75		0,72	0,25
residuals	0,257	-0,046	-0,538		0,576	-0,250
	0,0221	0,0007	0,0965		0,1108	0,0208
s^2	0,2508					
studentized residual	0,66	-0,11	-1,24		1,36	-0,99
	0,66	0,11	1,24		1,36	0,99
	1	1	1	0	1	1
	4,0E-06	5,9E-06	8,9E-06		2,0E-05	3,0E-05
	0,034	0,216	0,452		4,294	5,923
slope	245515,99350	**4,07E-06**				
intercept	-1,19270					
r^2	0,9749					

second tier r2: 0,9749

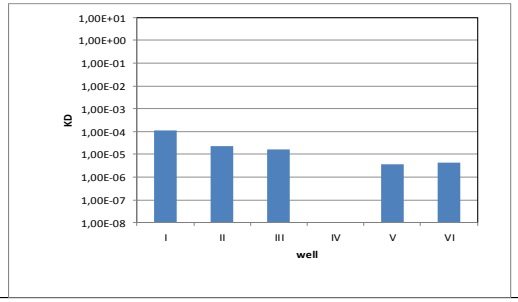
skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,833	4,111	3,444	3,167	0,944	0,722
[B] in [M]	3,951	5,926	8,889	13,333	20,000	30,000
[AB] in [M]	0,167	0,889	1,556	1,833	4,056	4,278
KD	1,1E-04	2,3E-05	1,6E-05		3,7E-06	4,3E-06
	3,1E-05					
SE(KD)	1,991E-05					
fb	0,154	0,462	0,552		0,843	0,822
	56,7%					
SE(fb)	12,707%					
n	5					
	1,10E-04	2,33E-05	1,62E-05	1,00E-12	3,71E-06	4,34E-06



4.4. PPB – Auswertung

Transil^{XL} PPB Binding Kit

Version 2 Revision 0

required input
optional input

Assay Plate Parameters

Lot Number:	FNRS
Date:	19.07.13
Assay volume:	240,0 µl
HSA + AGP concentration:	140,0 µM
φ:	1,80

Quality Control Parameters		
Saturation Threshold	99,9%	compound bound
Outlier Threshold	1,5	standard deviations
Threshold for outlier detection	0,98	r^2
Max difference of references:	30%	
MS saturation threshold	100000000	
model choice threshold	0,8000	r^2

	Compound name	sample volume [µL]	stock conc. [µM]					
		15,0 µl	80,0 µM	k_D	fb		r^2	TQI[1]
No 1	Doxorubicin	15,0 µl	80,0 µM	3,11E-04	66,3%	± 3,98%	0,9181	9,3
No 2	SL 5	15,0 µl	80,0 µM	6,07E-05	91,0%		1,0000	6,1
No 3	SL 4	15,0 µl	80,0 µM	5,49E-06	99,1%	± 0,04%	0,9903	7,6
No 4	SL 6	15,0 µl	80,0 µM	2,67E-05	95,8%	± 0,42%	0,9925	8,6
No 5	SL 7	15,0 µl	80,0 µM	1,46E-05	97,7%	± 0,30%	0,9755	7,6
No 6	SL 14	15,0 µl	80,0 µM	1,73E-05	97,2%	± 0,19%	0,9968	9,6
No 7	SL 1	15,0 µl	80,0 µM	3,96E-05	93,9%	± 0,70%	0,9955	9,6
No 8	SL 8	15,0 µl	80,0 µM	6,83E-06	98,9%	± 0,15%	0,9906	9,6
No 9	SL 12	15,0 µl	80,0 µM	5,47E-06	99,1%	± 0,06%	0,9978	8,3
No 10	SL 9	15,0 µl	80,0 µM	3,47E-05	94,6%	± 0,37%	0,9919	9,6
No 11	SL 2	15,0 µl	80,0 µM	2,29E-05	96,4%	± 0,25%	0,9975	9,5
No 12	SL 3	15,0 µl	80,0 µM	1,95E-05	96,9%	± 0,33%	0,9726	7,4
No 13	SL 13	15,0 µl	80,0 µM	6,43E-06	99,0%	± 0,08%	0,9797	7,4
No 14	SL 11	15,0 µl	80,0 µM	8,19E-05	88,2%	± 0,88%	0,9987	9,7
No 15	SL 10	15,0 µl	80,0 µM	1,02E-05	98,4%	± 0,14%	0,9835	8,6

Model Parmeters

1. Plasma protein binding

Physiological HSA conc.	5,88E-04
Physiological AGP conc.	2,45E-05

test article No 1 **Doxorubicin**

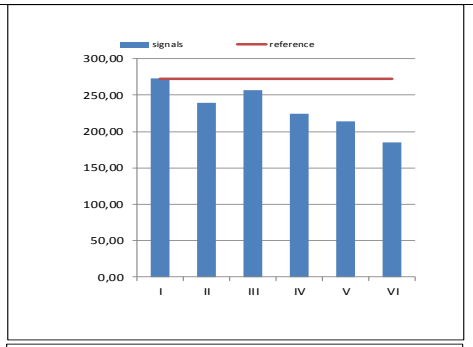
		5,00 µM				0 nm / amu	
adj. Ref 1		267,70	R		av ref too low		
Ref 2		270,50	ref diff ok		max ref too low R		
Signal Reference:		272,50	272,50	272,50	272,50	272,50	272,50	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		272,50	239,10	256,60	224,00	213,80	184,90	ok

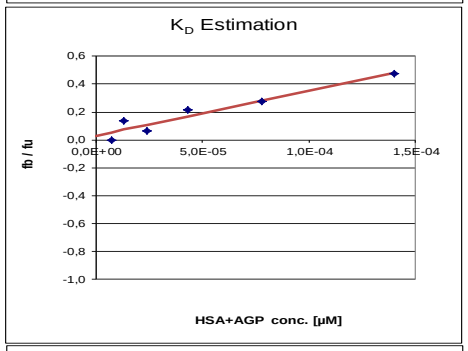
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

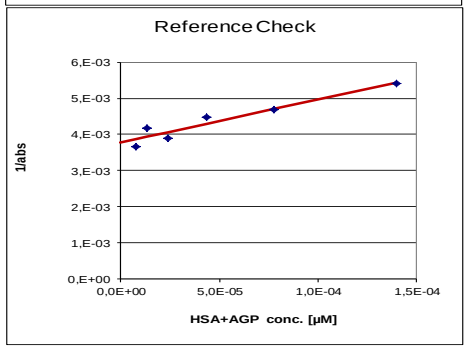
QC index: 9,3 **k_D= 3,11E-04** **fb= 66,3% ± 3,978%** **r^2= 0,91807**

warning messages:

		consistency check					
signal < reference	5	0	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,67E-03	4,18E-03	3,90E-03	4,46E-03	4,68E-03	5,41E-03
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	12,3%	5,8%	17,8%	21,5%	32,1%
(1-fu)/fu		0,000	0,140	0,062	0,217	0,275	0,474
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,140	0,062	0,217	0,275	0,474
slope		3212,55303					
intercept		0,03072					
r^2		0,9181					
prediction: (1-fu)/fu		3,9E+03	3,9E+03	3,9E+03	3,9E+03	3,9E+03	3,9E+03
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,000	0,140	0,062	0,217	0,275	0,474
slope		3212,55303					
intercept		0,03072					
r^2		0,9181					
prediction		0,055	0,074	0,108	0,170	0,281	0,480
residuals		-0,055	0,066	-0,046	0,047	-0,006	-0,007
standarized residuals		0,000	1,365	0,000	0,970	0,000	0,000
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		0,000	0,000	0,000	0,000	0,000	0,000
		0,000	0,140	0,062	0,217	0,275	0,474
slope		3212,55303	**3,11E-04**				
intercept		0,03072					
r^2		0,9181					
prediction	0,031	0,055	0,074	0,108	0,170	0,281	0,480
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,61	0,29	0,89	1,08	1,61
free protein con. [µM]		7,41	12,72	23,71	42,32	76,70	138,39
free protein con. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,2E-05	7,7E-05	1,4E-04
binding sites covered [%]		0,0%	4,6%	1,2%	2,1%	1,4%	1,1%
warning?							







reg	**3,11E-04 ± 0,72**	**0,91807**	
avg	**2,48E-04 ± 0,72**		1
choice	**3,11E-04 ± 0,72**		

value of "-5.0" places outlier markers below visible chart area

reference QC

protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,7E-03	4,2E-03	3,9E-03	4,5E-03	4,7E-03	5,4E-03
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal		3,7E-03	4,2E-03	3,9E-03	4,5E-03	4,7E-03	5,4E-03
slope		11,78919					
intercept		0,00378					
r^2		0,9181					
predicted 1/signal	0	3,9E-03	3,9E-03	4,1E-03	4,3E-03	4,7E-03	5,4E-03
Predicted Reference Value		264,38					
Actual Reference Value		272,50					
Relative deviation		3,0%					

QC index: 9,3

no. data points	10	reference	10	r^2 MA prediction	7	ref. Treatment	10	ref > signal	8	consistency	10
		10,0%	10	0,9999		A		6		6	10
6	10	20,0%	9	0,999		M		5	8	5	
5		50,0%	8	0,99		R	10	4		4	
4		100,0%	7	0,9	7	slopes	10	3		3	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2	
2		500,0%	3	0,7	5	:(lipid)/c(buffer) >0	10	1		intercept	
1		<500%		0,6	4			0		0,03	10
0				0,5	3						
				<0.5							

warning messages

data points
reference
ref. Treatment
ref > signal
consistency
r^2 MA prediction
slopes
intercept
binding
summary

1. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	-0,055	0,066	-0,046	0,047	-0,006	-0,007
	0,0023					
	0,0007	0,0011	0,0005	0,0006	0,0000	0,0000
s^2	0,0029					
studentized residual	-1,22	1,44	-0,96	0,95	-0,13	-0,27
	1,22	1,44	0,96	0,95	0,13	0,27
	1	1	1	1	1	1
	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	0,140	0,062	0,217	0,275	0,474
slope	3212,55303					
intercept	0,03072					
r^2	0,9181					

initial r2: 0,9181

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1
protein conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000
(1-fu)/fu	0,0	0,140	0,062	0,217	0,275	0,474
	3212,55303 w/o outlier		with outlier			
	0,02471	239,975	239,969	0,00 ok		
	0,9181 improvement?		no	1,0		
	accept outlier:		**na**			
abs(studentized residuals)	1,22	1,44	0,96	0,95	0,13	0,27	1,44
	7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05	7,77778E-05	0,00014	
	0,0		0,1	0,2	0,3	0,5	

2. step outlier detection

mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	-0,055	0,066	-0,046	0,047	-0,006	-0,007
	0,0007	0,0011	0,0005	0,0006	0,0000	0,0000
s^2	0,0029					
studentized residual	-1,22	1,44	-0,96	0,95	-0,13	-0,27
	1,22	1,44	0,96	0,95	0,13	0,27
	1	1	1	1	1	1
	0,000	0,000	0,000	0,000	0,000	0,000
	0,000	0,140	0,062	0,217	0,275	0,474
slope	3212,55303	**3,11E-04**				
intercept	0,03072					
r^2	0,9181					

second tier r2: 0,9181

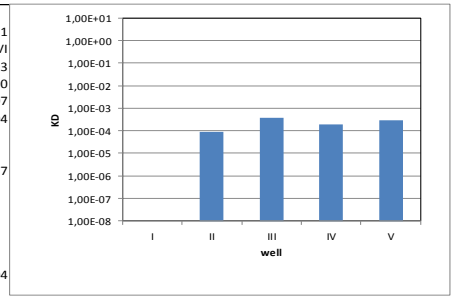
skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,387	4,708	4,110	3,923	3,393
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	0,613	0,292	0,890	1,077	1,607
KD		9,1E-05	3,8E-04	2,0E-04	2,8E-04	2,9E-04
	2,5E-04					
SE(KD)	4,493E-05					
fb		0,871	0,615	0,758	0,687	0,677
	72,2%					
SE(fb)	3,978%					
n	5					
		9,11E-05	3,83E-04	1,95E-04	2,79E-04	2,92E-04



cccc

test article No 2 **SL 5**

	5,00 µM					0 nm / amu	
adj. Ref 1	11,20	R		av ref too low			
Ref 2	9,00	ref diff ok		max ref too low	R		
Signal Reference:	17,00	17,00	17,00	17,00	17,00	17,00	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	17,00	14,10 na	na	na	na	na	ok

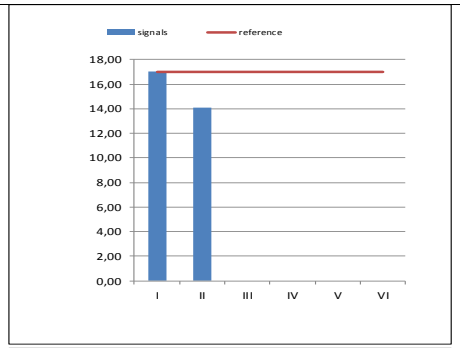
	missing values					
	1	1	0	0	0	0
	outlier detection					
consistency	ok	ok	**inconsistent**	**inconsistent**	**inconsistent**	**inconsistent**
saturation	ok	ok	**na**	**na**	**na**	**na**
statistics	ok	ok	**na**	**na**	**na**	**na**

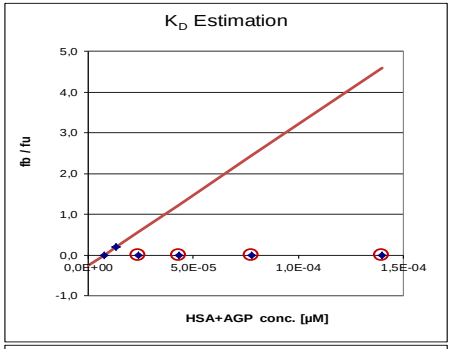
r^2= **1,00000**

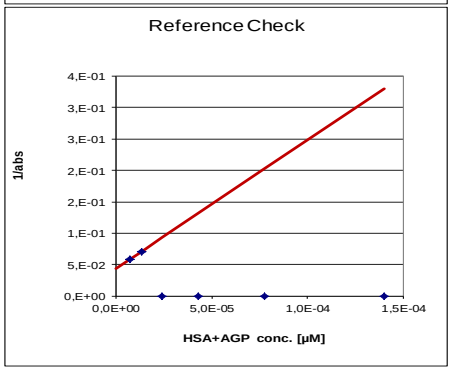
QC index: **6,1** k_D= **6,07E-05** **fb=** **91,0%**

warning messages: few valid data points, references exceed signal: solubility problem? data not consistent,

		consistency check					
signal < reference	1	0	1	0	0	0	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	2	1	1	0	0	0	0
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		5,88E-02	7,09E-02	#WERT!	#WERT!	#WERT!	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		0,0%	17,1%	0,0%	0,0%	0,0%	0,0%
(1-fu)/fu		0,000	0,206	0,000	0,000	0,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,000	0,206	0,000	0,000	0,000	0,000
slope		-607,04314					
intercept		0,06521					
r^2		0,1332					
prediction: (1-fu)/fu		-7,3E+02	-7,3E+02	-7,3E+02	-7,3E+02	-7,3E+02	-7,3E+02
outlier due to saturation and consistency		1	1	0	0	0	0
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05				
(1-fu)/fu		0,000	0,206				
slope		34699,51368					
intercept		-0,25709					
r^2		1,0000					
prediction		0,000	0,206				
residuals		0,000	0,000				
standarized residuals		0,000	0,000				
Threshold		1,5					
statistical outlier	2	1	1	0	0	0	0
		0,000	0,000				
		0,000	0,206				
slope		34699,51368	**2,88E-05**				
intercept		-0,25709					
r^2		1,0000					
prediction	-0,257	0,000	0,206	0,576	1,242	2,442	4,601
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	0,000	0,000	0,000	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,00	0,85	0,00	0,00	0,00	0,00
free protein con. [µM]		7,41	12,48	24,01	43,21	77,78	140,00
free protein con. [M]	0	7,4E-06	1,2E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
binding sites covered [%]		0,0%	6,4%	0,0%	0,0%	0,0%	0,0%
warning?							







reg	**2,88E-05 ± 0,91**	**1,00000**
avg	**6,07E-05 ± 0,91**	0
choice	**6,07E-05 ± 0,91**	

value of "-5.0" places outlier markers below visible chart area

reference QC

protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		5,9E-02	7,1E-02	#WERT!	#WERT!	#WERT!	#WERT!
valid protein conc. [µM]		7,4E-06	1,3E-05				
valid 1/signal		5,9E-02	7,1E-02				
slope		2041,14786					
intercept		0,04370					
r^2		1,0000					
predicted 1/signal	0	5,9E-02	7,1E-02	9,3E-02	1,3E-01	2,0E-01	3,3E-01
Predicted Reference Value		22,88					
Actual Reference Value		17,00					
Relative deviation		25,7%					

QC index: 6,1

no. data points	0	reference	8	r^2 MA prediction	10	ref. Treatment	10	ref > signal	1	consistency	0
		10,0%		0,9999	10	A		6		6	
6		20,0%		0,999	9	M		5		5	
5		50,0%	8	0,99	8	R	10	4		4	
4		100,0%	7	0,9	7	slopes	10	3		3	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2	
2	0	500,0%	3	0,7	5	(lipid)/c(buffer) >0	10	1	1	intercept	
1		<500%		0,6	4			0		-0,26	10
0				0,5	3						
				<0.5							

warning messages

data points few valid da	X
reference	
ref. Treatment	
ref > signal references e	X
consistency data not cor	X
r^2 MA prediction	
slopes	
intercept	
binding	

summary few valid data points, references exceed signal: solubility proble

1. step outlier detection

mean x	0,000		n	2		
$(x_i\text{-mean}(x))^2$	8,8E-12	8,8E-12				
$S(x_i\text{-mean}(x))^2$	1,8E-11					
	0,00	0,00				
residuals	0,000	0,000				
	0,0000					
	#DIV/0!	#DIV/0!				
s^2	#DIV/0!					
studentized residual	#DIV/0!	#DIV/0!				
	#DIV/0!	#DIV/0!				
	1	1	0	0	0	0
	0,000	0,000				
	0,000	0,206				
slope	34699,51368					
intercept	-0,25709					
r^2	1,0000					

2. step outlier detection

mean x	0,000		n	2		
$(x_i\text{-mean}(x))^2$	0,00	0,00				
$S(x_i\text{-mean}(x))^2$	0,00					
	0,00	0,00				
residuals	0,000	0,000				
	#DIV/0!	#DIV/0!				
s^2	#DIV/0!					
studentized residual	#DIV/0!	#DIV/0!				
	#DIV/0!	#DIV/0!				
	1	1	0	0	0	0
	0,000	0,000				
	0,000	0,206				
slope	34699,51368	**2,88E-05**				
intercept	-0,25709					
r^2	1,0000					

initial r2: 1,0000

skip outlier detection if r2 > 0,98

omit outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	0	0	0	0
protein conc. [µM]	0,000	0,000				
(1-fu)/fu	0,0	0,206				
	34699,51368	w/o outlier	with outlier			
	-0,25709	240,257	240,257	0,00	ok	
	1,0000	improvement	no	#DIV/0!		
		accept outlier	**#DIV/0!**			
abs(studentized residuals)	#DIV/0!	#DIV/0!				#DIV/0!
	#DIV/0!	#DIV/0!				
	#DIV/0!	#DIV/0!				

second tier r2: 1,0000

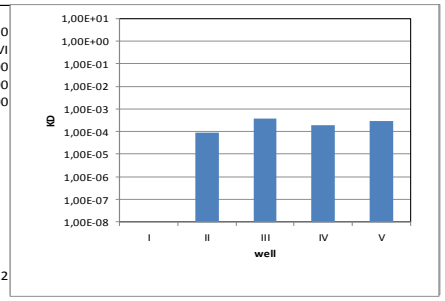
skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

Standard Error

	1	1	0	0	0	0
	I	II	III	IV	V	VI
[A] in [M]	5,000	4,147	5,000	5,000	5,000	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,000	0,853	0,000	0,000	0,000	0,000
KD		6,1E-05				
	6,1E-05					
SE(KD)						
fb		0,910				
	91,0%					
SE(fb)						
n	1					
		6,07E-05	1,00E-12	1,00E-12	1,00E-12	1,00E-12



test article No 3 **SL 4**

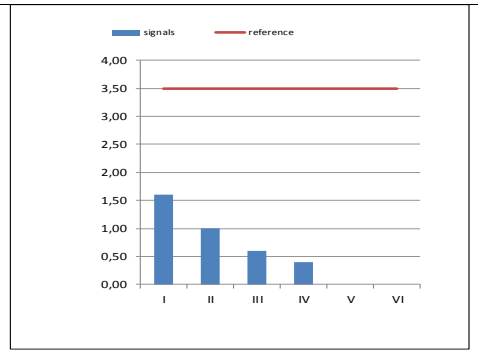
		5,00 µM				0 nm / amu	
adj. Ref 1		3,40	A		av ref ok		
Ref 2		3,60	ref diff ok		max ref ok		
Signal Reference:		3,50	3,50	3,50	3,50	3,50	3,50
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		1,60	1,00	0,60	0,40 na	na	ok

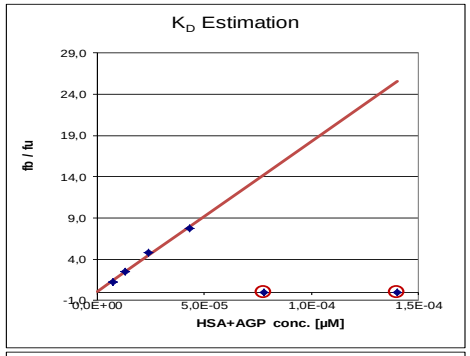
	missing values					
	1	1	1	1	0	0
	outlier detection					
consistency	ok	ok	ok	ok	**inconsistent**	**inconsistent**
saturation	ok	ok	ok	ok	**na**	**na**
statistics	ok	ok	ok	ok	**na**	**na**

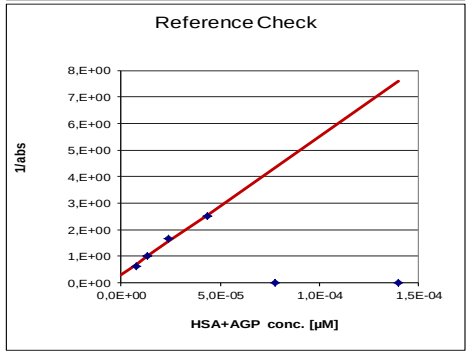
QC index: 7,6 **k_D= 5,49E-06** **fb= 99,1% ± 0,041%** **r^2= 0,99026**

warning messages: references exceed signal: solubility problem? data not consistent,

		consistency check					
signal < reference	4	1	1	1	1	0	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	4	1	1	1	1	0	0
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,25E-01	1,00E+00	1,67E+00	2,50E+00	#WERT!	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		54,3%	71,4%	82,9%	88,6%	0,0%	0,0%
(1-fu)/fu		1,188	2,500	4,833	7,750	0,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		1,188	2,500	4,833	7,750	0,000	0,000
slope		-26365,73197					
intercept		4,05531					
r^2		0,1887					
prediction: (1-fu)/fu		-3,2E+04	-3,2E+04	-3,2E+04	-3,2E+04	-3,2E+04	-3,2E+04
outlier due to saturation and consistency		1	1	1	1	0	0
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
(1-fu)/fu		1,188	2,500	4,833	7,750		
slope		182309,55544					
intercept		0,05868					
r^2		0,9903					
prediction		1,409	2,490	4,435	7,936		
residuals		-0,222	0,010	0,398	-0,186		
standarized residuals		0,000	0,035	1,400	0,000		
Threshold		1,5					
statistical outlier	4	1	1	1	1	0	0
		0,000	0,000	0,000	0,000		
		1,188	2,500	4,833	7,750		
slope		182309,55544	**5,49E-06**				
intercept		0,05868					
r^2		0,9903					
prediction	0,059	1,409	2,490	4,435	7,936	14,238	25,582
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	0,000	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,71	3,57	4,14	4,43	0,00	0,00
free protein con. [µM]		4,69	9,76	19,86	38,78	77,78	140,00
free protein con. [M]	0	4,7E-06	9,8E-06	2,0E-05	3,9E-05	7,8E-05	1,4E-04
binding sites covered [%]		36,6%	26,8%	17,3%	10,2%	0,0%	0,0%
warning?							







reg	**5,49E-06 ± 0,99**	**0,99026**	
avg	**4,24E-06 ± 0,99**		1
choice	**5,49E-06 ± 0,99**		

value of "-5.0" places outlier markers below visible chart area

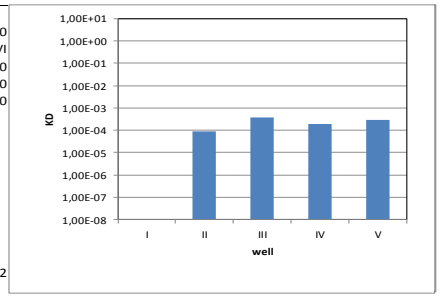
		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,3E-01	1,0E+00	1,7E+00	2,5E+00	#WERT!	#WERT!
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
valid 1/signal		6,3E-01	1,0E+00	1,7E+00	2,5E+00		
slope		52088,44441					
intercept		0,30248					
r^2		0,9903					
predicted 1/signal	0	6,9E-01	1,0E+00	1,6E+00	2,6E+00	4,4E+00	7,6E+00
Predicted Reference Value		3,31					
Actual Reference Value		3,50					
Relative deviation		5,5%					

QC index: 7,6

no. data points	6	reference	10	r^2 MA predicl	8	ref. Treatment	10	ref > signal	6	consistency	3	warning messages											
		10,0%	10	0,9999		A	10	6		6		data points											
6		20,0%	9	0,999		M		5		5		reference											
5		50,0%	8	0,99	8	R		4	6	4	3	ref. Treatment											
4	6	100,0%	7	0,9	7	slopes	10	3		3		ref > signal	references exceed	X									
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency	data not consisten	X									
2		500,0%	3	0,7	5	:(lipid)/c(buffer) >0	10	1		intercept		r^2 MA prediction											
1		<500%		0,6	4			0		0,06	10	slopes											
0				0,5	3							intercept											
				<0.5								binding											
												summary	references exceed signal: solubility problem? data not consistent,										

	1. step outlier detection					
mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	2,1E-10	7,5E-11	4,1E-12	4,5E-10		
$S(x_i\text{-mean}(x))^2$	7,4E-10					
	0,46	0,65	0,74	0,14		
residuals	-0,222	0,010	0,398	-0,186		
	0,0809					
	0,0246	0,0000	0,0793	0,0173		
s^2	0,1213					
studentized residual	-0,94	0,04	1,33	-1,41		
	0,94	0,04	1,33	1,41		
	1	1	1	1	0	0
	0,000	0,000	0,000	0,000		
	1,188	2,500	4,833	7,750		
slope	182309,55544					
intercept	0,05868					
r^2	0,9903					
	2. step outlier detection					
mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00		
$S(x_i\text{-mean}(x))^2$	0,00					
	0,46	0,65	0,74	0,14		
residuals	-0,222	0,010	0,398	-0,186		
	0,0246	0,0000	0,0793	0,0173		
s^2	0,1213					
studentized residual	-0,94	0,04	1,33	-1,41		
	0,94	0,04	1,33	1,41		
	1	1	1	1	0	0
	0,000	0,000	0,000	0,000		
	1,188	2,500	4,833	7,750		
slope	182309,55544	**5,49E-06**				
intercept	0,05868					
r^2	0,9903					

	Standard Error					
	1	1	1	1	0	0
	I	II	III	IV	V	VI
[A] in [M]	2,286	1,429	0,857	0,571	5,000	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,714	3,571	4,143	4,429	0,000	0,000
KD	4,0E-06	3,9E-06	4,1E-06	5,0E-06		
	4,2E-06					
SE(KD)	2,573E-07					
fb	0,994	0,994	0,993	0,992		
	99,3%					
SE(fb)	0,041%					
n	4					
	3,95E-06	3,91E-06	4,11E-06	5,00E-06	1,00E-12	1,00E-12



initial r2:	0,9903					
skip outlier detection if r2 >	0,98					
	omit	outlier detection				
check, if estimation of reference assay volume becomes worse after outlier detection						
	checking effect of outlier removal on intercept estimation of nt/c					
	1	1	1	1	0	0
protein conc. [µM]	0,000	0,000	0,000	0,000		
(1-fu)/fu	1,2	2,500	4,833	7,750		
	182309,55544	w/o outlier	with outlier			
	0,05868	239,941	239,941	0,00	ok	
	0,9903	improvement?	no	1,0		
		accept outlier:	**na**			
abs(studentized residuals)	0,94	0,04	1,33	1,41		1,41
	7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05		
	1,2	2,5	4,8			
second tier r2:	0,9903					
skip outlier detection if r2 >	0,98					
	omit	outlier detection				
no outliers will be recorded if there are only 3 or less points left at step 1 or 2						

test article No 4 **SL 6**

		5,00 µM				0 nm / amu	
adj. Ref 1		102,00	A		av ref ok		
Ref 2		76,20	ref diff ok		max ref ok		
Signal Reference:		89,10	89,10	89,10	89,10	89,10	89,10
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		52,40	42,60	34,50	25,30	17,20	13,10	ok

-

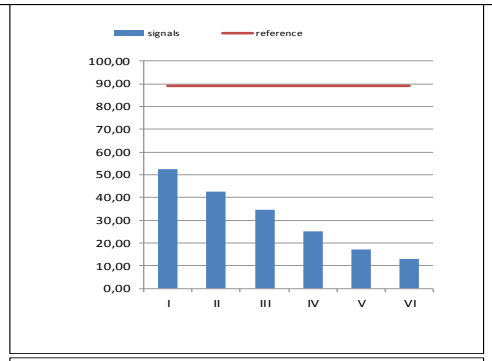
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	**outlier**	ok

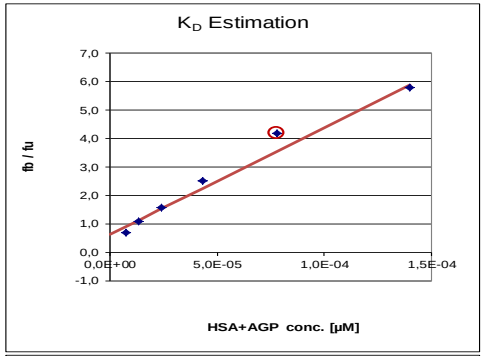
r^2= **0,99245**

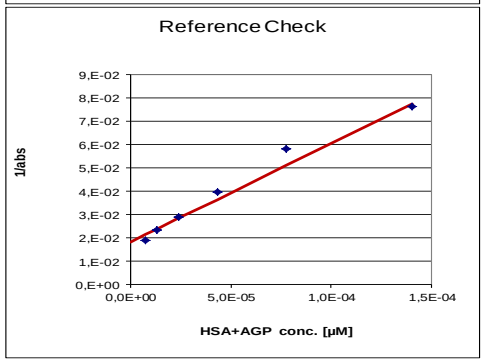
QC index: **8,6** **k_D= 2,67E-05** **fb=** **95,8% ± 0,425%**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,91E-02	2,35E-02	2,90E-02	3,95E-02	5,81E-02	7,63E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		41,2%	52,2%	61,3%	71,6%	80,7%	85,3%
(1-fu)/fu		0,700	1,092	1,583	2,522	4,180	5,802
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,700	1,092	1,583	2,522	4,180	5,802
slope		38831,86913					
intercept		0,66761					
r^2		0,9777					
prediction: (1-fu)/fu		4,7E+04	4,7E+04	4,7E+04	4,7E+04	4,7E+04	4,7E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,700	1,092	1,583	2,522	4,180	5,802
slope		38831,86913					
intercept		0,66761					
r^2		0,9777					
prediction		0,955	1,185	1,600	2,346	3,688	6,104
residuals		-0,255	-0,094	-0,017	0,176	0,492	-0,303
standarized residuals		0,000	0,000	0,000	0,595	1,661	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	1	0	1
		0,000	0,000	0,000	0,000		0,000
		0,700	1,092	1,583	2,522		5,802
slope		37498,25056	**2,67E-05**				
intercept		0,62993					
r^2		0,9925					
prediction	0,630	0,908	1,130	1,530	2,250	3,546	5,880
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	4,180	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,06	2,61	3,06	3,58	4,03	4,26
free protein con. [µM]		5,35	10,73	20,94	39,63	73,74	135,74
free protein con. [M]	0	5,3E-06	1,1E-05	2,1E-05	4,0E-05	7,4E-05	1,4E-04
binding sites covered [%]		27,8%	19,6%	12,8%	8,3%	5,2%	3,0%
warning?							







reg	**2,67E-05 ± 0,98**	**0,99245**	
avg	**1,40E-05 ± 0,98**		1
choice	**2,67E-05 ± 0,98**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,9E-02	2,3E-02	2,9E-02	4,0E-02	5,8E-02	7,6E-02
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		1,4E-04
valid 1/signal		1,9E-02	2,3E-02	2,9E-02	4,0E-02		7,6E-02
slope		420,85579					
intercept		0,01829					
r^2		0,9925					
predicted 1/signal	0	2,1E-02	2,4E-02	2,8E-02	3,6E-02	5,1E-02	7,7E-02
Predicted Reference Value		54,66					
Actual Reference Value		89,10					
Relative deviation		38,6%					

QC index: 8,6

no. data points	9	reference	8	r^2 MA predic	8	ref. Treatment	10	ref > signal	10	consistency	10											warning messages
		10,0%		0,9999		A	10	6	10	6	10											data points
6		20,0%		0,999		M		5		5												reference
5	9	50,0%	8	0,99	8	R		4		4												ref. Treatment
4		100,0%	7	0,9	7	slopes	10	3		3												ref > signal
3		200,0%	5	0,8	6	1/abs > 0	5	2		2												consistency
2		500,0%	3	0,7	5	pid)/c(buffer) >0	10	1		intercept												r^2 MA prediction
1		<500%		0,6	4			0		0,63	5											slopes
0				0,5	3																	intercept
				<0.5																		binding
																						summary

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	-0,255	-0,094	-0,017	0,176	0,492	-0,303
	0,0878					
	0,0162	0,0022	0,0001	0,0078	0,0606	0,0229
s^2	0,1098					
studentized residual	-0,93	-0,33	-0,06	0,58	1,69	-1,99
	0,93	0,33	0,06	0,58	1,69	1,99
	1	1	1	1	0	1
	0,000	0,000	0,000	0,000		0,000
	0,700	1,092	1,583	2,522		5,802
slope	37498,25056					
intercept	0,62993					
r^2	0,9925					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00		0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,68	0,71	0,76	0,80		0,05
residuals	-0,207	-0,038	0,053	0,272		-0,078
	0,0143	0,0005	0,0009	0,0246		0,0020
s^2	0,0424					
studentized residual	-1,22	-0,22	0,29	1,48		-1,70
	1,22	0,22	0,29	1,48		1,70
	1	1	1	1	0	1
	0,000	0,000	0,000	0,000		0,000
	0,700	1,092	1,583	2,522		5,802
slope	37498,25056	**2,67E-05**				
intercept	0,62993					
r^2	0,9925					

initial r2: 0,9777

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	0	1
protein conc. [µM]	0,000	0,000	0,000	0,000		0,000
(1-fu)/fu	0,7	1,092	1,583	2,522		5,802
	37498,25056	w/o outlier	with outlier			
	0,37605	239,624	239,332	0,00	ok	
	0,9925	improvement?	no	3,0		
		accept outlier:	**no**			
abs(studentized residuals)	0,93	0,33	0,06	0,58	1,99	0,93
	7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05	0,00014	
		1,1	1,6	2,5	5,8	

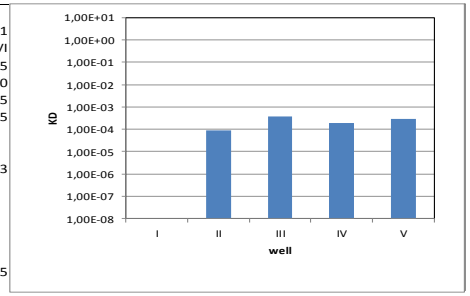
second tier r2: 0,9925

skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	1	1	1	1	0	1
	I	II	III	IV	V	VI
[A] in [M]	2,941	2,391	1,936	1,420	0,965	0,735
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,059	2,609	3,064	3,580	4,035	4,265
KD	7,6E-06	9,8E-06	1,3E-05	1,6E-05		2,3E-05
	1,4E-05					
SE(KD)	2,736E-06					
fb	0,988	0,984	0,979	0,975		0,963
	97,8%					
SE(fb)	0,425%					
n	5					
	7,64E-06	9,83E-06	1,32E-05	1,57E-05	1,00E-12	2,34E-05



	test article No		5	**SL 7**				
		5,00 µM					0 nm / amu	
adj. Ref 1		5,60	A		av ref ok			
Ref 2		6,50	ref diff ok		max ref ok			
Signal Reference:		6,05	6,05	6,05	6,05	6,05	6,05	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		2,70	1,70	1,20	0,70	0,80	0,50	ok

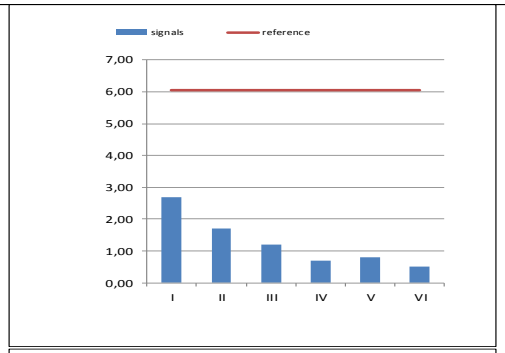
-

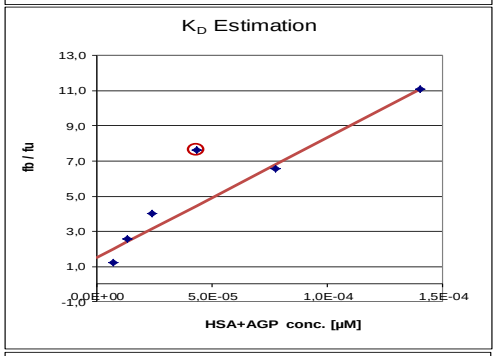
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	**outlier**	ok	ok

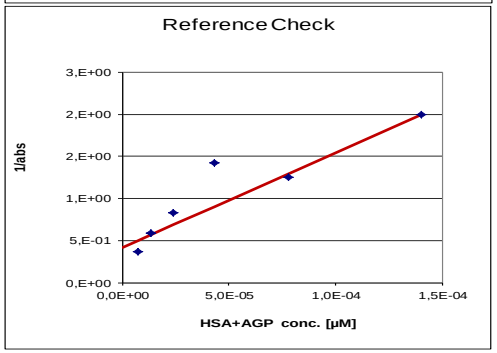
QC index: 7,6 **k_D= 1,46E-05** **fb= 97,7% ± 0,297%** **r^2= 0,97551**

warning messages: poor intercept fit,

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,70E-01	5,88E-01	8,33E-01	1,43E+00	1,25E+00	2,00E+00
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		55,4%	71,9%	80,2%	88,4%	86,8%	91,7%
(1-fu)/fu		1,241	2,559	4,042	7,643	6,563	11,100
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		1,241	2,559	4,042	7,643	6,563	11,100
slope		66370,82141					
intercept		2,14241					
r^2		0,8508					
prediction: (1-fu)/fu		8,0E+04	8,0E+04	8,0E+04	8,0E+04	8,0E+04	8,0E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		1,241	2,559	4,042	7,643	6,563	11,100
slope		66370,82141					
intercept		2,14241					
r^2		0,8508					
prediction		2,634	3,028	3,736	5,010	7,305	11,434
residuals		-1,393	-0,469	0,306	2,633	-0,742	-0,334
standarized residuals		0,000	0,000	0,218	1,876	0,000	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	0	1	1
		0,000	0,000	0,000		0,000	0,000
		1,241	2,559	4,042		6,563	11,100
slope		68301,68740	**1,46E-05**				
intercept		1,51451					
r^2		0,9755					
prediction	1,515	2,021	2,425	3,154	4,466	6,827	11,077
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	7,643	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,77	3,60	4,01	4,42	4,34	4,59
free protein con. [µM]		4,64	9,74	20,00	38,79	73,44	135,41
free protein con. [M]	0	4,6E-06	9,7E-06	2,0E-05	3,9E-05	7,3E-05	1,4E-04
binding sites covered [%]		37,4%	27,0%	16,7%	10,2%	5,6%	3,3%
warning?							







reg	**1,46E-05 ± 0,99**	**0,97551**	
avg	**7,18E-06 ± 0,99**		1
choice	**1,46E-05 ± 0,99**		

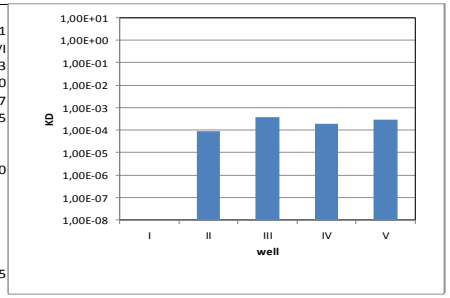
value of "-5.0" places outlier markers below visible chart area

		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,7E-01	5,9E-01	8,3E-01	1,4E+00	1,3E+00	2,0E+00
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05		7,8E-05	1,4E-04
valid 1/signal		3,7E-01	5,9E-01	8,3E-01		1,3E+00	2,0E+00
slope		11289,53511					
intercept		0,41562					
r^2		0,9755					
predicted 1/signal	0	5,0E-01	5,7E-01	6,9E-01	9,0E-01	1,3E+00	2,0E+00
Predicted Reference Value		2,41					
Actual Reference Value		6,05					
Relative deviation		60,2%					

				QC index: 7,6								warning messages	
no. data points	9	reference	7	r^2 MA predict	7	ref. Treatment	10	ref > signal	10	consistency	10	data points	
		10,0%		0,9999		A	10	6	10	6	10	reference	
6		20,0%		0,999		M		5		5		ref. Treatment	
5	9	50,0%		0,99		R		4		4		ref > signal	
4		100,0%	7	0,9	7	slopes	10	3		3		consistency	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		r^2 MA prediction	
2		500,0%	3	0,7	5	ipid)/c(buffer) >0	10	1		intercept		slopes	
1		<500%		0,6	4			0		1,51	0	intercept poor interce	X
0				0,5	3							binding	
				<0.5								summary poor intercept fit,	

	1. step outlier detection															
mean x	0,000		n	6												
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09			initial r2:		0,8508					
$S(x_i\text{-mean}(x))^2$	1,3E-08								skip outlier detection if r2 >		0,98					
	0,68	0,72	0,78	0,83	0,78	0,21					consider	outlier detection				
residuals	-1,393	-0,469	0,306	2,633	-0,742	-0,334										
	1,9696								check, if estimation of reference assay volume becomes worse after outlier detection							
	0,4854	0,0549	0,0234	1,7326	0,1377	0,0279										
s^2	2,4620									checking effect of outlier removal on intercept estimation of nt/c						
studentized residual	-1,07	-0,35	0,22	1,84	-0,54	-0,46				1	1	1	0	1	1	
	1,07	0,35	0,22	1,84	0,54	0,46			protein conc. [µM]	0,000	0,000	0,000		0,000	0,000	
	1	1	1	0	1	1			(1-fu)/fu	1,2	2,559	4,042		6,563	11,100	
	0,000	0,000	0,000		0,000	0,000				68301,68740	w/o outlier	with outlier				
	1,241	2,559	4,042		6,563	11,100				1,53379	238,466	237,858	0,00	ok		
slope	68301,68740									0,9755	improvement?	no	6,1			
intercept	1,51451										accept outlier:	**no**				
r^2	0,9755								abs(studentized residuals)	1,07	0,35	0,22		0,54	0,46	1,07
	2. step outlier detection									7,4091E-06	1,33364E-05	2,40055E-05		7,77778E-05	0,00014	
mean x	0,000		n	5							2,6	4,0		6,6	11,1	
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00		0,00	0,00										
$S(x_i\text{-mean}(x))^2$	0,00															
	0,64	0,68	0,74		0,75	0,20										
residuals	-0,780	0,133	0,888		-0,264	0,023			second tier r2:		0,9755					
	0,2027	0,0059	0,2626		0,0233	0,0002			skip outlier detection if r2 >		0,98					
s^2	0,4947										consider	outlier detection				
studentized residual	-1,39	0,23	1,47		-0,43	0,07										
	1,39	0,23	1,47		0,43	0,07			no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
	1	1	1	0	1	1										
	0,000	0,000	0,000		0,000	0,000										
	1,241	2,559	4,042		6,563	11,100										
slope	68301,68740	**1,46E-05**														
intercept	1,51451															
r^2	0,9755															

	Standard Error					
	1	1	1	0	1	1
	I	II	III	IV	V	VI
[A] in [M]	2,231	1,405	0,992	0,579	0,661	0,413
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,769	3,595	4,008	4,421	4,339	4,587
KD	3,7E-06	3,8E-06	4,9E-06		1,1E-05	1,2E-05
	7,2E-06					
SE(KD)	1,864E-06					
fb	0,994	0,994	0,992		0,982	0,980
	98,8%					
SE(fb)	0,297%					
n	5					
	3,74E-06	3,81E-06	4,95E-06	1,00E-12	1,12E-05	1,22E-05



test article No 6 **SL 14**

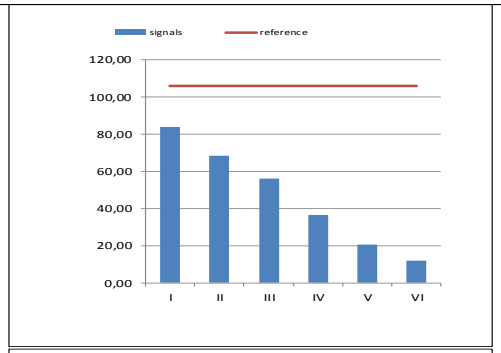
		5,00 µM				0 nm / amu	
adj. Ref 1		101,90	A		av ref ok		
Ref 2		109,70	ref diff ok		max ref ok		
Signal Reference:		105,80	105,80	105,80	105,80	105,80	105,80
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		83,90	68,30	56,20	36,40	20,70	12,00 ok
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		ok	ok	ok	ok	ok	ok

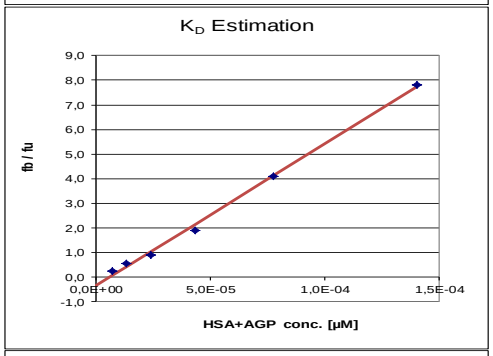
r^2= **0,99675**

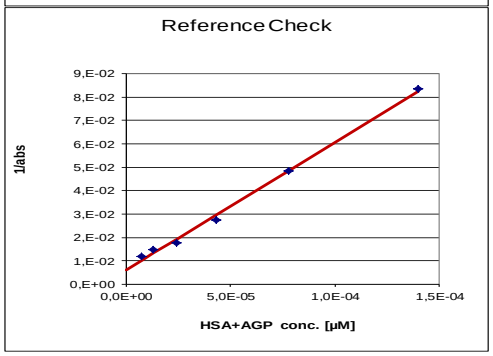
QC index: **9,6** **k_D= 1,73E-05** **fb= 97,2% ± 0,191%**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,19E-02	1,46E-02	1,78E-02	2,75E-02	4,83E-02	8,33E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		20,7%	35,4%	46,9%	65,6%	80,4%	88,7%
(1-fu)/fu		0,261	0,549	0,883	1,907	4,111	7,817
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,261	0,549	0,883	1,907	4,111	7,817
slope		57720,34630					
intercept		-0,35339					
r^2		0,9968					
prediction: (1-fu)/fu		6,9E+04	6,9E+04	6,9E+04	6,9E+04	6,9E+04	6,9E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,261	0,549	0,883	1,907	4,111	7,817
slope		57720,34630					
intercept		-0,35339					
r^2		0,9968					
prediction		0,074	0,416	1,032	2,141	4,136	7,727
residuals		0,187	0,133	-0,150	-0,234	-0,025	0,089
standarized residuals		1,123	0,798	0,000	0,000	0,000	0,536
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		0,000	0,000	0,000	0,000	0,000	0,000
		0,261	0,549	0,883	1,907	4,111	7,817
slope		57720,34630	**1,73E-05**				
intercept		-0,35339					
r^2		0,9968					
prediction	-0,353	0,074	0,416	1,032	2,141	4,136	7,727
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		1,03	1,77	2,34	3,28	4,02	4,43
free protein con. [µM]		6,37	11,56	21,66	39,93	73,76	135,57
free protein con. [M]	0	6,4E-06	1,2E-05	2,2E-05	4,0E-05	7,4E-05	1,4E-04
binding sites covered [%]		14,0%	13,3%	9,8%	7,6%	5,2%	3,2%
warning?							







reg	**1,73E-05 ± 0,97**	**0,99675**	
avg	**2,10E-05 ± 0,97**		1
choice	**1,73E-05 ± 0,97**		

value of "-5.0" places outlier markers below visible chart area

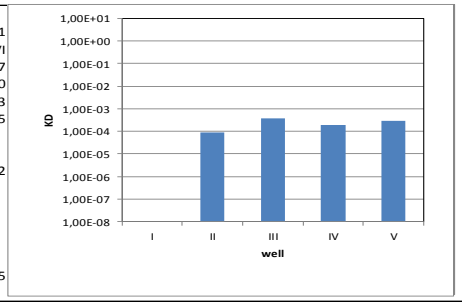
		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,2E-02	1,5E-02	1,8E-02	2,7E-02	4,8E-02	8,3E-02
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal		1,2E-02	1,5E-02	1,8E-02	2,7E-02	4,8E-02	8,3E-02
slope		545,56093					
intercept		0,00611					
r^2		0,9968					
predicted 1/signal	0	1,0E-02	1,3E-02	1,9E-02	3,0E-02	4,9E-02	8,2E-02
Predicted Reference Value		163,62					
Actual Reference Value		105,80					
Relative deviation		35,3%					

QC index: 9,6

no. data points	10	reference	8	r^2 MA predict	8	ref. Treatment	10	ref > signal	10	consistency	10							warning messages
		10,0%		0,9999		A	10	6	10	6	10							data points
6	10	20,0%		0,999		M		5		5								reference
5		50,0%	8	0,99	8	R		4		4								ref. Treatment
4		100,0%	7	0,9	7	slopes	10	3		3								ref > signal
3		200,0%	5	0,8	6	1/abs > 0	5	2		2								consistency
2		500,0%	3	0,7	5	ł)/c(buffer) >0	10	1		intercept								r^2 MA prediction
1		<500%		0,6	4			0		-0,35	10							slopes
0				0,5	3													intercept
				<0.5														binding
																		summary

		1. step outlier detection															
mean x		0,000		n	6												
$(x_i\text{-mean}(x))^2$		1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09		initial r2:		0,9968						
$S(x_i\text{-mean}(x))^2$		1,3E-08							skip outlier detection if r2 >		0,98						
		0,68	0,72	0,78	0,83	0,78	0,21				omit	outlier detection					
residuals		0,187	0,133	-0,150	-0,234	-0,025	0,089										
		0,0277							check, if estimation of reference assay volume becomes worse after outlier detection								
		0,0087	0,0044	0,0056	0,0137	0,0002	0,0020										
s^2		0,0346										checking effect of outlier removal on intercept estimation of nt/c					
studentized residual		1,21	0,84	-0,91	-1,38	-0,15	1,04			1	1	1	1	1	1		
		1,21	0,84	0,91	1,38	0,15	1,04		protein conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000		
		1	1	1	1	1	1		(1-fu)/fu	0,3	0,549	0,883	1,907	4,111	7,817		
		0,000	0,000	0,000	0,000	0,000	0,000			57720,34630	w/o outlier	with outlier					
		0,261	0,549	0,883	1,907	4,111	7,817			-0,27346	240,273	240,353	0,00	ok			
slope		57720,34630								0,9968	improvement?	yes	1,0				
intercept		-0,35339									accept outlier:	**na**					
r^2		0,9968							abs(studentized residuals)	1,21	0,84	0,91	1,38	0,15	1,04	1,38	
		2. step outlier detection								7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05	7,77778E-05	0,00014		
mean x		0,000		n	6					0,3	0,5	0,9		4,1	7,8		
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00	0,00	0,00										
$S(x_i\text{-mean}(x))^2$		0,00															
		0,68	0,72	0,78	0,83	0,78	0,21										
residuals		0,187	0,133	-0,150	-0,234	-0,025	0,089		second tier r2:		0,9968						
		0,0087	0,0044	0,0056	0,0137	0,0002	0,0020		skip outlier detection if r2 >		0,98						
s^2		0,0346									omit	outlier detection					
studentized residual		1,21	0,84	-0,91	-1,38	-0,15	1,04										
		1,21	0,84	0,91	1,38	0,15	1,04		no outliers will be recorded if there are only 3 or less points left at step 1 or 2								
		1	1	1	1	1	1										
		0,000	0,000	0,000	0,000	0,000	0,000										
		0,261	0,549	0,883	1,907	4,111	7,817										
slope		57720,34630	**1,73E-05**														
intercept		-0,35339															
r^2		0,9968															

	Standard Error					
	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	3,965	3,228	2,656	1,720	0,978	0,567
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	1,035	1,772	2,344	3,280	4,022	4,433
KD	2,4E-05	2,1E-05	2,5E-05	2,1E-05	1,8E-05	1,7E-05
	2,1E-05					
SE(KD)	1,251E-06					
fb	0,962	0,967	0,961	0,967	0,972	0,972
	96,7%					
SE(fb)	0,191%					
n	6					
	2,44E-05	2,11E-05	2,45E-05	2,09E-05	1,79E-05	1,73E-05



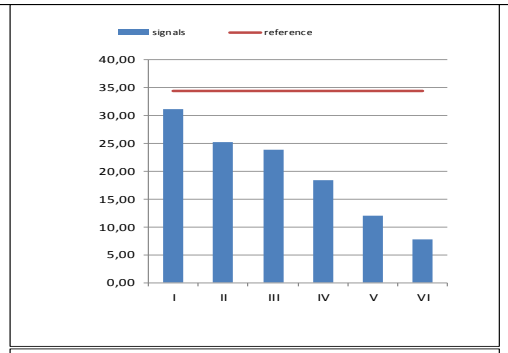
	test article No	7	**SL 1**				
	5,00 µM					0 nm / amu	
adj. Ref 1	34,40	M		av ref too low			
Ref 2	23,10	high ref diff	M	max ref ok			
Signal Reference:	34,40	34,40	34,40	34,40	34,40	34,40	
TRANSIL well:	I	II	III	IV	V	VI	
Signal in TRANSIL wells:	31,20	25,20	23,90	18,40	12,00	7,70	ok

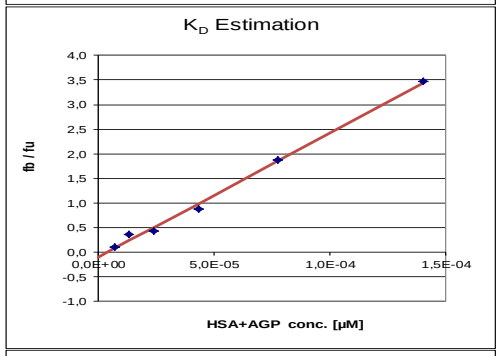
	Ref 1 <> Ref 2					
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	ok	ok

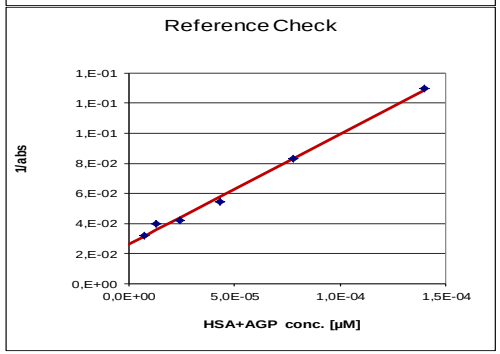
QC index: **9,6** **k_D= 3,96E-05** **fb= 93,9% ± 0,698%** **r^2= 0,99548**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,21E-02	3,97E-02	4,18E-02	5,43E-02	8,33E-02	1,30E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		9,3%	26,7%	30,5%	46,5%	65,1%	77,6%
(1-fu)/fu		0,103	0,365	0,439	0,870	1,867	3,468
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,103	0,365	0,439	0,870	1,867	3,468
slope		25266,52552					
intercept		-0,10237					
r^2		0,9955					
prediction: (1-fu)/fu		3,0E+04	3,0E+04	3,0E+04	3,0E+04	3,0E+04	3,0E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,103	0,365	0,439	0,870	1,867	3,468
slope		25266,52552					
intercept		-0,10237					
r^2		0,9955					
prediction		0,085	0,235	0,504	0,989	1,863	3,435
residuals		0,018	0,130	-0,065	-0,120	0,004	0,033
standarized residuals		0,206	1,517	0,000	0,000	0,045	0,379
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		0,000	0,000	0,000	0,000	0,000	0,000
		0,103	0,365	0,439	0,870	1,867	3,468
slope		25266,52552	**3,96E-05**				
intercept		-0,10237					
r^2		0,9955					
prediction	-0,102	0,085	0,235	0,504	0,989	1,863	3,435
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,47	1,34	1,53	2,33	3,26	3,88
free protein con. [µM]		6,94	12,00	22,48	40,88	74,52	136,12
free protein con. [M]	0	6,9E-06	1,2E-05	2,2E-05	4,1E-05	7,5E-05	1,4E-04
binding sites covered [%]		6,3%	10,0%	6,4%	5,4%	4,2%	2,8%
warning?							







reg	**3,96E-05 ± 0,93**	**0,99548**
avg	**4,63E-05 ± 0,93**	1
choice	**3,96E-05 ± 0,93**	

value of "-5.0" places outlier markers below visible chart area

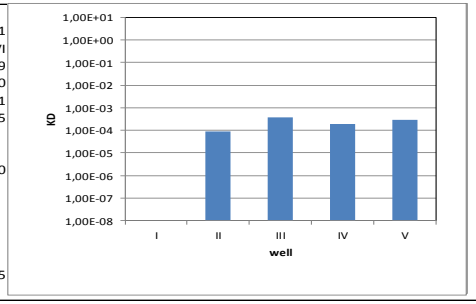
		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		3,2E-02	4,0E-02	4,2E-02	5,4E-02	8,3E-02	1,3E-01
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal		3,2E-02	4,0E-02	4,2E-02	5,4E-02	8,3E-02	1,3E-01
slope		734,49202					
intercept		0,02609					
r^2		0,9955					
predicted 1/signal	0	3,2E-02	3,6E-02	4,4E-02	5,8E-02	8,3E-02	1,3E-01
Predicted Reference Value		38,32					
Actual Reference Value		34,40					
Relative deviation		10,2%					

QC index: 9,6

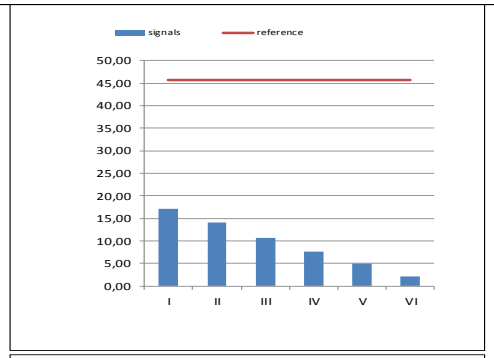
no. data points	10	reference	9	r^2 MA predict	8	ref. Treatment	10	ref > signal	10	consistency	10	warning messages		
		10,0%		0,9999		A		6	10	6	10	data points		
6	10	20,0%	9	0,999		M	10	5		5		reference		
5		50,0%	8	0,99	8	R		4		4		ref. Treatment		
4		100,0%	7	0,9	7	slopes	10	3		3		ref > signal		
3		200,0%	5	0,8	6	1/abs > 0	5	2		2		consistency		
2		500,0%	3	0,7	5	id)/c(buffer) >0	10	1		intercept		r^2 MA prediction		
1		<500%		0,6	4			0		-0,10	10	slopes		
0				0,5	3							intercept		
				<0.5								binding		
												summary		

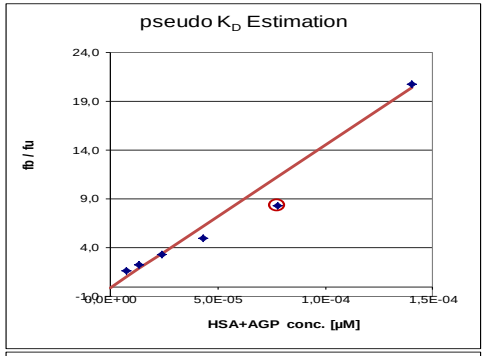
	1. step outlier detection														
mean x	0,000		n	6											
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09		initial r2:	0,9955						
$S(x_i\text{-mean}(x))^2$	1,3E-08							skip outlier detection if r2 >	0,98						
	0,68	0,72	0,78	0,83	0,78	0,21			omit	outlier detection					
residuals	0,018	0,130	-0,065	-0,120	0,004	0,033									
	0,0074							check, if estimation of reference assay volume becomes worse after outlier detection							
	0,0001	0,0043	0,0011	0,0036	0,0000	0,0003									
s^2	0,0092								checking effect of outlier removal on intercept estimation of nt/c						
studentized residual	0,22	1,60	-0,77	-1,37	0,05	0,74			1	1	1	1	1	1	
	0,22	1,60	0,77	1,37	0,05	0,74		protein conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000	
	1	1	1	1	1	1		(1-fu)/fu	0,1	0,365	0,439	0,870	1,867	3,468	
	0,000	0,000	0,000	0,000	0,000	0,000			25266,52552	w/o outlier	with outlier				
	0,103	0,365	0,439	0,870	1,867	3,468			-0,07317	240,073	240,102	0,00	ok		
slope	25266,52552								0,9955	improvement	yes	1,0			
intercept	-0,10237									accept outlier	**na**				
r^2	0,9955							abs(studentized residuals)	0,22	1,60	0,77	1,37	0,05	0,74	1,60
	2. step outlier detection								7,4091E-06		2,40055E-05	4,32099E-05	7,77778E-05	0,00014	
mean x	0,000		n	6					0,1		0,4	0,9	1,9	3,5	
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00									
$S(x_i\text{-mean}(x))^2$	0,00														
	0,68	0,72	0,78	0,83	0,78	0,21									
residuals	0,018	0,130	-0,065	-0,120	0,004	0,033		second tier r2:	0,9955						
	0,0001	0,0043	0,0011	0,0036	0,0000	0,0003		skip outlier detection if r2 >	0,98						
s^2	0,0092								omit	outlier detection					
studentized residual	0,22	1,60	-0,77	-1,37	0,05	0,74									
	0,22	1,60	0,77	1,37	0,05	0,74		no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
	1	1	1	1	1	1									
	0,000	0,000	0,000	0,000	0,000	0,000									
	0,103	0,365	0,439	0,870	1,867	3,468									
slope	25266,52552	**3,96E-05**													
intercept	-0,10237														
r^2	0,9955														

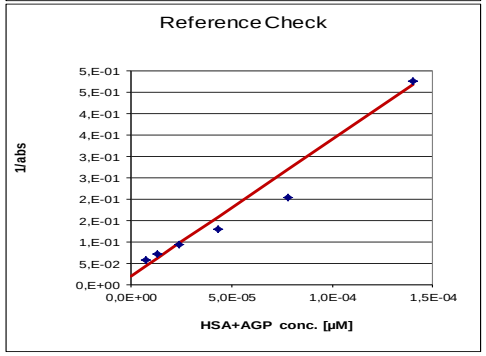
	Standard Error					
	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,535	3,663	3,474	2,674	1,744	1,119
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,465	1,337	1,526	2,326	3,256	3,881
KD	6,8E-05	3,3E-05	5,1E-05	4,7E-05	4,0E-05	3,9E-05
	4,6E-05					
SE(KD)	5,010E-06					
fb	0,900	0,949	0,923	0,929	0,939	0,940
	93,0%					
SE(fb)	0,698%					
n	6					
	6,77E-05	3,29E-05	5,12E-05	4,70E-05	3,99E-05	3,93E-05



		test article No	8	**SL 8**				
		5,00 µM					0 nm / amu	
adj. Ref 1		47,10	A		av ref ok			
Ref 2		44,30	ref diff ok		max ref ok			
Signal Reference:		45,70	45,70	45,70	45,70	45,70	45,70	
TRANSIL well:		I	II	III	IV	V	VI	
Signal in TRANSIL wells:		17,20	14,00	10,60	7,70	4,90	2,10	ok
		-						
		missing values						
		1	1	1	1	1	1	
		outlier detection						
consistency		ok	ok	ok	ok	ok	ok	
saturation		ok	ok	ok	ok	ok	ok	
statistics		ok	ok	ok	ok	**outlier**	ok	
							r^2= 0,99055	
QC index:	**9,6**	**k_D= 6,83E-06**		**fb=**	**98,9% ± 0,148%**			
warning messages:								
		consistency check						
signal < reference	6	1	1	1	1	1	1	
binding > 0%		1	1	1	1	1	1	
steady increase in binding & summary	6	1	1	1	1	1	1	
		removal of outliers due to saturation						
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04	
1/signal in TRANSIL well		5,81E-02	7,14E-02	9,43E-02	1,30E-01	2,04E-01	4,76E-01	
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2	
fb in assay		62,4%	69,4%	76,8%	83,2%	89,3%	95,4%	
(1-fu)/fu		1,657	2,264	3,311	4,935	8,327	20,762	
saturation threshold		99,999%						
systematic outlier (saturation)		1	1	1	1	1	0	
consistent saturation		1	1	1	1	1	1	
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04	
(1-fu)/fu		1,657	2,264	3,311	4,935	8,327	20,762	
slope		140217,34004						
intercept		-0,26896						
r^2		0,9642						
prediction: (1-fu)/fu		1,7E+05	1,7E+05	1,7E+05	1,7E+05	1,7E+05	1,7E+05	
outlier due to saturation and consistency		1	1	1	1	1	1	
		statistical outlier detection & removal						
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000	
(1-fu)/fu		1,657	2,264	3,311	4,935	8,327	20,762	
slope		140217,34004						
intercept		-0,26896						
r^2		0,9642						
prediction		0,770	1,601	3,097	5,790	10,637	19,361	
residuals		0,887	0,663	0,214	-0,855	-2,310	1,400	
standarized residuals		0,650	0,486	0,157	0,000	0,000	1,027	
Threshold		1,5						
statistical outlier	5	1	1	1	1	0	1	
		0,000	0,000	0,000	0,000		0,000	
		1,657	2,264	3,311	4,935		20,762	
slope		146474,94882	**6,83E-06**					
intercept		-0,09220						
r^2		0,9906						
prediction	-0,092	0,993	1,861	3,424	6,237	11,300	20,414	
		outlier						
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04	
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	8,327	-5,000	
		Binding QC						
no. of binding sites		1						
conc. protein-compound complex [µM]		3,12	3,47	3,84	4,16	4,46	4,77	
free protein con. [µM]		4,29	9,87	20,17	39,05	73,31	135,23	
free protein con. [M]	0	4,3E-06	9,9E-06	2,0E-05	3,9E-05	7,3E-05	1,4E-04	
binding sites covered [%]		42,1%	26,0%	16,0%	9,6%	5,7%	3,4%	
warning?								







reg	**6,83E-06 ± 0,99**	**0,99055**	
avg	**5,49E-06 ± 0,99**		1
choice	**6,83E-06 ± 0,99**		

value of "-5.0" places outlier markers below visible chart area

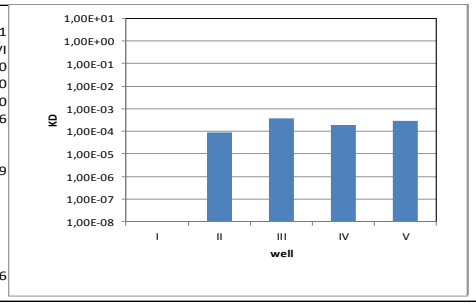
		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		5,8E-02	7,1E-02	9,4E-02	1,3E-01	2,0E-01	4,8E-01
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		1,4E-04
valid 1/signal		5,8E-02	7,1E-02	9,4E-02	1,3E-01		4,8E-01
slope		3205,14111					
intercept		0,01986					
r^2		0,9906					
predicted 1/signal	0	4,4E-02	6,3E-02	9,7E-02	1,6E-01	2,7E-01	4,7E-01
Predicted Reference Value		50,34					
Actual Reference Value		45,70					
Relative deviation		9,2%					

QC index: 9,6

no. data points	9	reference	10	r^2 MA predicl	8	ref. Treatment	10	ref > signal	10	consistency	10		warning messages
		10,0%	10	0,9999		A	10	6	10	6	10		data points
6		20,0%	9	0,999		M		5		5			reference
5	9	50,0%	8	0,99	8	R		4		4			ref. Treatment
4		100,0%	7	0,9	7	slopes	10	3		3			ref > signal
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			consistency
2		500,0%	3	0,7	5	id)/c(buffer) >0	10	1		intercept			r^2 MA prediction
1		<500%		0,6	4			0		-0,09	10		slopes
0				0,5	3								intercept
				<0.5									binding
													summary

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,887	0,663	0,214	-0,855	-2,310	1,400
	1,8604					
	0,1967	0,1100	0,0115	0,1826	1,3344	0,4903
s^2	2,3255					
studentized residual	0,70	0,51	0,16	-0,62	-1,72	2,00
	0,70	0,51	0,16	0,62	1,72	2,00
	1	1	1	1	0	1
	0,000	0,000	0,000	0,000		0,000
	1,657	2,264	3,311	4,935		20,762
slope	146474,94882					
intercept	-0,09220					
r^2	0,9906					
	2. step outlier detection					
mean x	0,000		n	5		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00		0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,68	0,71	0,76	0,80		0,05
residuals	0,664	0,403	-0,113	-1,302		0,348
	0,1469	0,0541	0,0042	0,5650		0,0403
s^2	0,8106					
studentized residual	0,90	0,53	-0,14	-1,62		1,73
	0,90	0,53	0,14	1,62		1,73
	1	1	1	1	0	1
	0,000	0,000	0,000	0,000		0,000
	1,657	2,264	3,311	4,935		20,762
slope	146474,94882	**6,83E-06**				
intercept	-0,09220					
r^2	0,9906					

	Standard Error					
	1	1	1	1	0	1
	I	II	III	IV	V	VI
[A] in [M]	1,882	1,532	1,160	0,842	0,536	0,230
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	3,118	3,468	3,840	4,158	4,464	4,770
KD	2,6E-06	4,4E-06	6,1E-06	7,9E-06		6,5E-06
	5,5E-06					
SE(KD)	9,212E-07					
fb	0,996	0,993	0,990	0,987		0,989
	99,1%					
SE(fb)	0,148%					
n	5					
	2,59E-06	4,36E-06	6,09E-06	7,91E-06	1,00E-12	6,51E-06



initial r2: 0,9642

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	0	1
protein conc. [µM]	0,000	0,000	0,000	0,000		0,000
(1-fu)/fu	1,7	2,264	3,311	4,935		20,762
	146474,94882 w/o outlier		with outlier			
	1,03690	238,963	240,269	-0,01 ok		
	0,9906 improvement?		yes	3,8		
	accept outlier:		**yes**			
abs(studentized residuals)	0,70	0,51	0,16	0,62		2,00	0,70
	7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05		0,00014
		2,3	3,3	4,9		20,8

second tier r2: 0,9906

skip outlier detection if r2 > 0,98

omit outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

test article No 9 **SL 12**

		5,00 µM				0 nm / amu	
adj. Ref 1		22,00	A		av ref ok		
Ref 2		19,60	ref diff ok		max ref ok		
Signal Reference:		20,80	20,80	20,80	20,80	20,80	20,80
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		8,50	5,60	3,60	2,30	1,90 na	ok

-

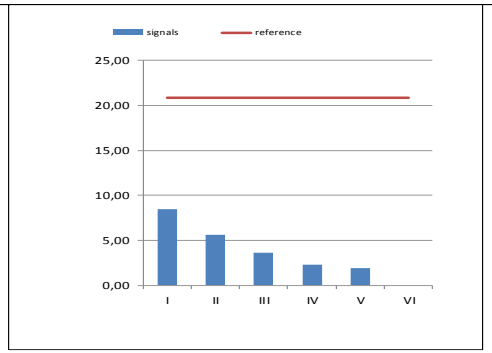
	missing values					
	1	1	1	1	1	0
	outlier detection					
consistency	ok	ok	ok	ok	ok	**inconsistent**
saturation	ok	ok	ok	ok	ok	**na**
statistics	ok	ok	ok	ok	**outlier**	**na**

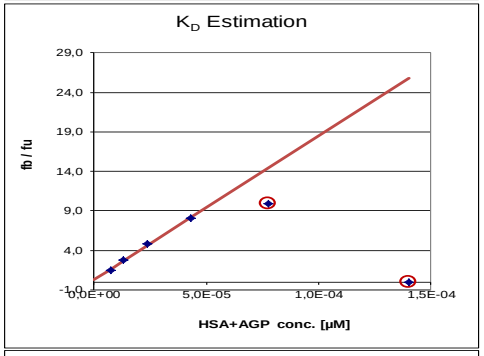
r^2= **0,99784**

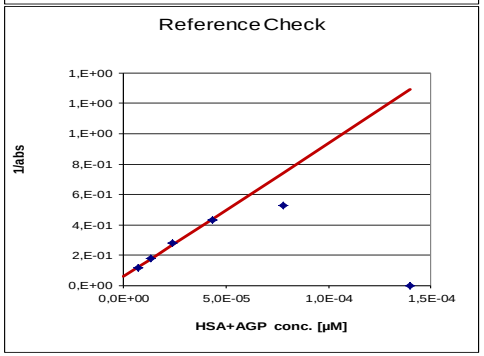
QC index: **8,3** **k_D= 5,47E-06** **fb=** **99,1% ± 0,061%**

warning messages: data not consistent,

		consistency check					
signal < reference	5	1	1	1	1	1	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	1	1	0
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,18E-01	1,79E-01	2,78E-01	4,35E-01	5,26E-01	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		59,1%	73,1%	82,7%	88,9%	90,9%	0,0%
(1-fu)/fu		1,447	2,714	4,778	8,043	9,947	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		1,447	2,714	4,778	8,043	9,947	0,000
slope		-7013,91244					
intercept		4,84573					
r^2		0,0084					
prediction: (1-fu)/fu		-8,4E+03	-8,4E+03	-8,4E+03	-8,4E+03	-8,4E+03	-8,4E+03
outlier due to saturation and consistency		1	1	1	1	1	0
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
(1-fu)/fu		1,447	2,714	4,778	8,043	9,947	
slope		120740,03901					
intercept		1,38374					
r^2		0,9257					
prediction		2,278	2,994	4,282	6,601	10,775	
residuals		-0,831	-0,280	0,496	1,443	-0,827	
standarized residuals		0,000	0,000	0,510	1,484	0,000	
Threshold		1,5					
statistical outlier	4	1	1	1	1	0	0
		0,000	0,000	0,000	0,000		
		1,447	2,714	4,778	8,043		
slope		182951,88675	**5,47E-06**				
intercept		0,22250					
r^2		0,9978					
prediction	0,222	1,578	2,662	4,614	8,128	14,452	25,836
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	9,947	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,96	3,65	4,13	4,45	4,54	0,00
free protein con. [µM]		4,45	9,68	19,87	38,76	73,23	140,00
free protein con. [M]	0	4,5E-06	9,7E-06	2,0E-05	3,9E-05	7,3E-05	1,4E-04
binding sites covered [%]		39,9%	27,4%	17,2%	10,3%	5,8%	0,0%
warning?							







reg	**5,47E-06 ± 0,99**	**0,99784**	
avg	**3,91E-06 ± 0,99**		1
choice	**5,47E-06 ± 0,99**		

value of "-5.0" places outlier markers below visible chart area

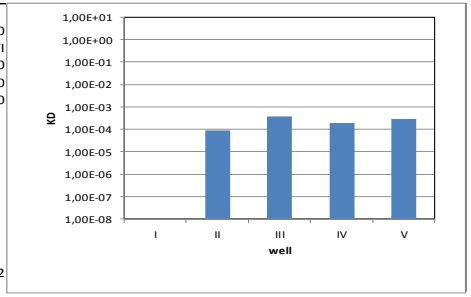
		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,2E-01	1,8E-01	2,8E-01	4,3E-01	5,3E-01	#WERT!
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
valid 1/signal		1,2E-01	1,8E-01	2,8E-01	4,3E-01		
slope		8795,76379					
intercept		0,05877					
r^2		0,9978					
predicted 1/signal	0	1,2E-01	1,8E-01	2,7E-01	4,4E-01	7,4E-01	1,3E+00
Predicted Reference Value		17,01					
Actual Reference Value		20,80					
Relative deviation		18,2%					

QC index: 8,3

no. data points	6	reference	9	r^2 MA predict	8	ref. Treatment	10	ref > signal	8	consistency	6		warning messages			
		10,0%		0,9999		A	10	6		6			data points			
6		20,0%	9	0,999		M		5	8	5	6		reference			
5		50,0%	8	0,99	8	R		4		4			ref. Treatment			
4	6	100,0%	7	0,9	7	slopes	10	3		3			ref > signal			
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			consistency	data not consisten	X	
2		500,0%	3	0,7	5	ıid)/c(buffer) >0	10	1		intercept			r^2 MA prediction			
1		<500%		0,6	4			0		0,22	10		slopes			
0				0,5	3								intercept			
				<0.5									binding			
													summary	data not consistent,		

		1. step outlier detection														
mean x		0,000		n	5											
$(x_i\text{-mean}(x))^2$		6,6E-10	3,9E-10	8,4E-11	1,0E-10	2,0E-09				initial r2:		0,9257				
$S(x_i\text{-mean}(x))^2$		3,2E-09								skip outlier detection if r2 >		0,98				
		0,60	0,68	0,77	0,77	0,18						consider	outlier detection			
residuals		-0,831	-0,280	0,496	1,443	-0,827										
		0,9451								check, if estimation of reference assay volume becomes worse after outlier detection						
		0,2303	0,0261	0,0819	0,6937	0,2281										
s^2		1,2601										checking effect of outlier removal on intercept estimation of nt/c				
studentized residual		-0,96	-0,30	0,50	1,47	-1,72						1	1	1	1	0	0
		0,96	0,30	0,50	1,47	1,72				protein conc. [µM]		0,000	0,000	0,000	0,000		
		1	1	1	1	0	0			(1-fu)/fu		1,4	2,714	4,778	8,043		
		0,000	0,000	0,000	0,000							182951,88675	w/o outlier	with outlier			
		1,447	2,714	4,778	8,043							0,22250	239,778	238,616	0,00	ok	
slope		182951,88675										0,9978	improvement?	no	34,4		
intercept		0,22250											accept outlier:	**yes**			
r^2		0,9978								abs(studentized residuals)		0,96	0,30	0,50	1,47		1,47
		2. step outlier detection										7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05		
mean x		0,000		n	4							1,4	2,7	4,8			
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00												
$S(x_i\text{-mean}(x))^2$		0,00															
		0,46	0,65	0,74	0,14												
residuals		-0,131	0,052	0,163	-0,084					second tier r2:		0,9978					
		0,0086	0,0013	0,0134	0,0036					skip outlier detection if r2 >		0,98					
s^2		0,0268										omit	outlier detection				
studentized residual		-1,17	0,39	1,16	-1,36												
		1,17	0,39	1,16	1,36					no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
		1	1	1	1	0	0										
		0,000	0,000	0,000	0,000												
		1,447	2,714	4,778	8,043												
slope		182951,88675	**5,47E-06**														
intercept		0,22250															
r^2		0,9978															

	Standard Error					
	1	1	1	1	0	0
	I	II	III	IV	V	VI
[A] in [M]	2,043	1,346	0,865	0,553	0,457	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,957	3,654	4,135	4,447	4,543	0,000
KD	3,1E-06	3,6E-06	4,2E-06	4,8E-06		
	3,9E-06					
SE(KD)	3,764E-07					
fb	0,995	0,994	0,993	0,992		
	99,4%					
SE(fb)	0,061%					
n	4					
	3,08E-06	3,57E-06	4,16E-06	4,82E-06	1,00E-12	1,00E-12



test article No 10 **SL 9**

		5,00 µM				0 nm / amu	
adj. Ref 1		99,20	A		av ref ok		
Ref 2		89,00	ref diff ok		max ref ok		
Signal Reference:		94,10	94,10	94,10	94,10	94,10	94,10
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		80,80	68,50	53,60	36,90	22,40	18,70	ok

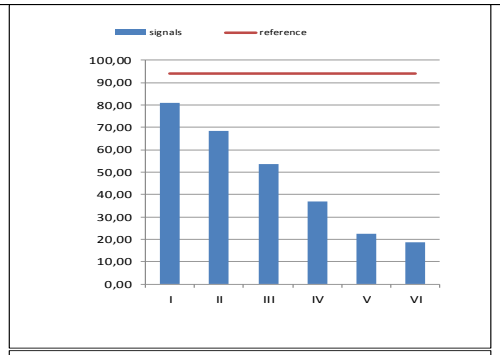
-

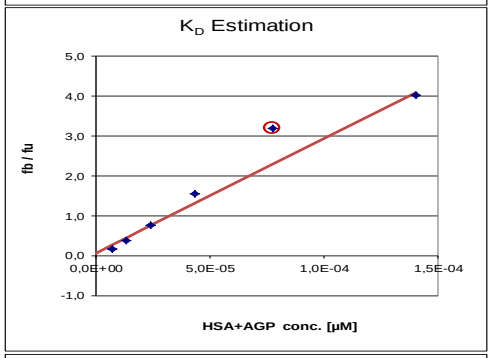
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		ok	ok	ok	ok	**outlier**	ok

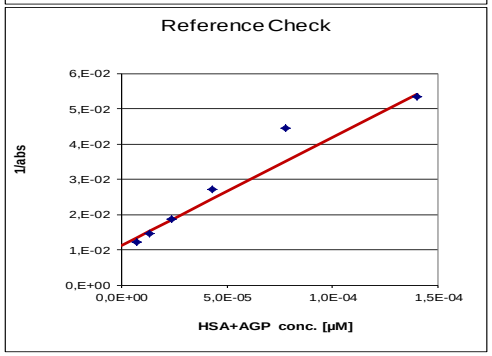
QC index: **9,6** **k_D= 3,47E-05** **fb=** **94,6% ± 0,367%** **r^2= 0,99188**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,24E-02	1,46E-02	1,87E-02	2,71E-02	4,46E-02	5,35E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		14,1%	27,2%	43,0%	60,8%	76,2%	80,1%
(1-fu)/fu		0,165	0,374	0,756	1,550	3,201	4,032
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,165	0,374	0,756	1,550	3,201	4,032
slope		30698,20517					
intercept		0,11524					
r^2		0,9443					
prediction: (1-fu)/fu		3,7E+04	3,7E+04	3,7E+04	3,7E+04	3,7E+04	3,7E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,165	0,374	0,756	1,550	3,201	4,032
slope		30698,20517					
intercept		0,11524					
r^2		0,9443					
prediction		0,343	0,525	0,852	1,442	2,503	4,413
residuals		-0,178	-0,151	-0,097	0,108	0,698	-0,381
standarized residuals		0,000	0,000	0,000	0,288	1,855	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	1	0	1
		0,000	0,000	0,000	0,000		0,000
		0,165	0,374	0,756	1,550		4,032
slope		28807,57066	**3,47E-05**				
intercept		0,06183					
r^2		0,9919					
prediction	0,062	0,275	0,446	0,753	1,307	2,302	4,095
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	3,201	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,71	1,36	2,15	3,04	3,81	4,01
free protein con. [µM]		6,70	11,98	21,85	40,17	73,97	135,99
free protein con. [M]	0	6,7E-06	1,2E-05	2,2E-05	4,0E-05	7,4E-05	1,4E-04
binding sites covered [%]		9,5%	10,2%	9,0%	7,0%	4,9%	2,9%
warning?							







reg	**3,47E-05 ± 0,95**	**0,99188**	
avg	**3,23E-05 ± 0,95**		1
choice	**3,47E-05 ± 0,95**		

value of "-5.0" places outlier markers below visible chart area

test article No 11 **SL 2**

		5,00 µM				0 nm / amu	
adj. Ref 1		31,90	A		av ref ok		
Ref 2		29,30	ref diff ok		max ref ok		
Signal Reference:		30,60	30,60	30,60	30,60	30,60	30,60
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		20,70	17,20	13,70	9,20	4,50	4,20	ok

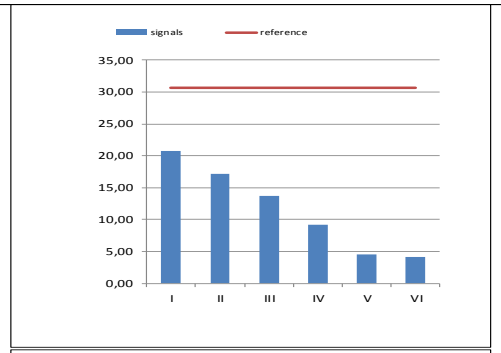
-

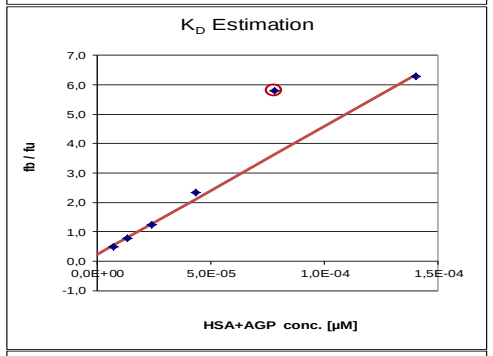
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	ok
saturation	ok	ok	ok	ok	ok	ok
statistics	ok	ok	ok	ok	**outlier**	ok

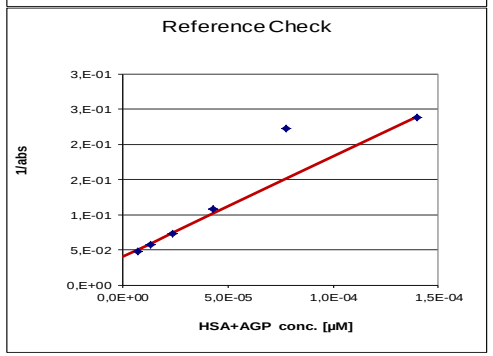
QC index: 9,5 **k_D= 2,29E-05** **fb= 96,4% ± 0,246%** **r^2= 0,99753**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		4,83E-02	5,81E-02	7,30E-02	1,09E-01	2,22E-01	2,38E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		32,4%	43,8%	55,2%	69,9%	85,3%	86,3%
(1-fu)/fu		0,478	0,779	1,234	2,326	5,800	6,286
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,478	0,779	1,234	2,326	5,800	6,286
slope		48160,86927					
intercept		0,36301					
r^2		0,8878					
prediction: (1-fu)/fu		5,8E+04	5,8E+04	5,8E+04	5,8E+04	5,8E+04	5,8E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,478	0,779	1,234	2,326	5,800	6,286
slope		48160,86927					
intercept		0,36301					
r^2		0,8878					
prediction		0,720	1,005	1,519	2,444	4,109	7,106
residuals		-0,242	-0,226	-0,286	-0,118	1,691	-0,820
standarized residuals		0,000	0,000	0,000	0,000	1,956	0,000
Threshold		1,5					
statistical outlier	5	1	1	1	1	0	1
		0,000	0,000	0,000	0,000		0,000
		0,478	0,779	1,234	2,326		6,286
slope		43580,29150	**2,29E-05**				
intercept		0,23362					
r^2		0,9975					
prediction	0,234	0,557	0,815	1,280	2,117	3,623	6,335
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	5,800	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		1,62	2,19	2,76	3,50	4,26	4,31
free protein con. [µM]		5,79	11,15	21,24	39,71	73,51	135,69
free protein con. [M]	0	5,8E-06	1,1E-05	2,1E-05	4,0E-05	7,4E-05	1,4E-04
binding sites covered [%]		21,8%	16,4%	11,5%	8,1%	5,5%	3,1%
warning?							







reg	**2,29E-05 ± 0,97**	**0,99753**	
avg	**1,65E-05 ± 0,97**		1
choice	**2,29E-05 ± 0,97**		

value of "-5.0" places outlier markers below visible chart area

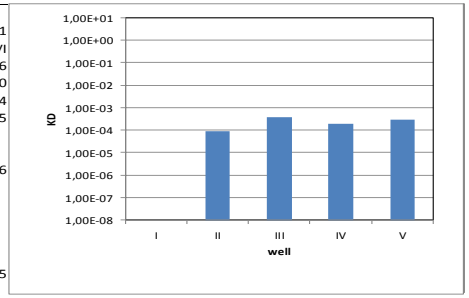
		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		4,8E-02	5,8E-02	7,3E-02	1,1E-01	2,2E-01	2,4E-01
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		1,4E-04
valid 1/signal		4,8E-02	5,8E-02	7,3E-02	1,1E-01		2,4E-01
slope		1424,19253					
intercept		0,04031					
r^2		0,9975					
predicted 1/signal	0	5,1E-02	5,9E-02	7,5E-02	1,0E-01	1,5E-01	2,4E-01
Predicted Reference Value		24,81					
Actual Reference Value		30,60					
Relative deviation		18,9%					

QC index: 9,5

no. data points	9	reference	9	r^2 MA predict	8	ref. Treatment	10	ref > signal	10	consistency	10		warning messages	
		10,0%		0,9999		A	10	6	10	6	10		data points	
6		20,0%	9	0,999		M		5		5			reference	
5	9	50,0%	8	0,99	8	R		4		4			ref. Treatment	
4		100,0%	7	0,9	7	slopes	10	3		3			ref > signal	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			consistency	
2		500,0%	3	0,7	5	id)/c(buffer) >0	10	1		intercept			r^2 MA prediction	
1		<500%		0,6	4			0		0,23	10		slopes	
0				0,5	3								intercept	
				<0.5									binding	
													summary	

		1. step outlier detection														
mean x		0,000		n	6											
$(x_i\text{-mean}(x))^2$		1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09		initial r2:		0,8878					
$S(x_i\text{-mean}(x))^2$		1,3E-08							skip outlier detection if r2 >		0,98					
		0,68	0,72	0,78	0,83	0,78	0,21				consider	outlier detection				
residuals		-0,242	-0,226	-0,286	-0,118	1,691	-0,820									
		0,7474							check, if estimation of reference assay volume becomes worse after outlier detection							
		0,0146	0,0128	0,0204	0,0035	0,7150	0,1680									
s^2		0,9343									checking effect of outlier removal on intercept estimation of nt/c					
studentized residual		-0,30	-0,28	-0,34	-0,13	1,99	-1,85			1	1	1	1	0	1	
		0,30	0,28	0,34	0,13	1,99	1,85		protein conc. [µM]	0,000	0,000	0,000	0,000		0,000	
		1	1	1	1	0	1		(1-fu)/fu	0,5	0,779	1,234	2,326		6,286	
		0,000	0,000	0,000	0,000		0,000			43580,29150	w/o outlier	with outlier				
		0,478	0,779	1,234	2,326		6,286			0,07398	239,926	239,637	0,00	ok		
slope		43580,29150								0,9975	improvement?	no	45,5			
intercept		0,23362									accept outlier:	**yes**				
r^2		0,9975							abs(studentized residuals)	0,30	0,28	0,34	0,13		1,85	0,34
		2. step outlier detection								7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05		0,00014	
mean x		0,000		n	5					0,5	0,8		2,3		6,3	
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00		0,00									
$S(x_i\text{-mean}(x))^2$		0,00														
		0,68	0,71	0,76	0,80		0,05									
residuals		-0,078	-0,036	-0,046	0,209		-0,049		second tier r2:		0,9975					
		0,0020	0,0004	0,0007	0,0146		0,0008		skip outlier detection if r2 >		0,98					
s^2		0,0186									omit	outlier detection				
studentized residual		-0,70	-0,31	-0,39	1,72		-1,61									
		0,70	0,31	0,39	1,72		1,61		no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
		1	1	1	1	0	1									
		0,000	0,000	0,000	0,000		0,000									
		0,478	0,779	1,234	2,326		6,286									
slope		43580,29150	**2,29E-05**													
intercept		0,23362														
r^2		0,9975														

	Standard Error					
	1	1	1	1	0	1
	I	II	III	IV	V	VI
[A] in [M]	3,382	2,810	2,239	1,503	0,735	0,686
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	1,618	2,190	2,761	3,497	4,265	4,314
KD	1,2E-05	1,4E-05	1,7E-05	1,7E-05		2,2E-05
	1,6E-05					
SE(KD)	1,594E-06					
fb	0,981	0,977	0,973	0,973		0,966
	97,4%					
SE(fb)	0,246%					
n	5					
	1,21E-05	1,43E-05	1,72E-05	1,71E-05	1,00E-12	2,16E-05



test article No 12 **SL 3**

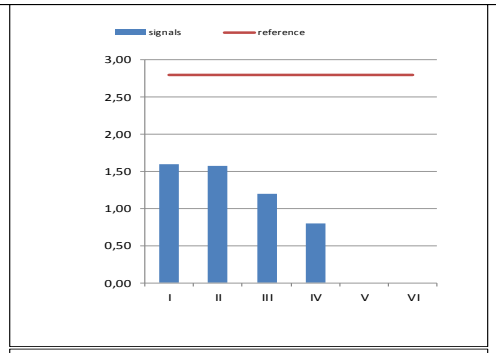
		5,00 µM				0 nm / amu	
adj. Ref 1		2,60	A		av ref ok		
Ref 2		3,00	ref diff ok		max ref ok		
Signal Reference:		2,80	2,80	2,80	2,80	2,80	2,80
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		1,60	1,57	1,20	0,80 na	na	ok

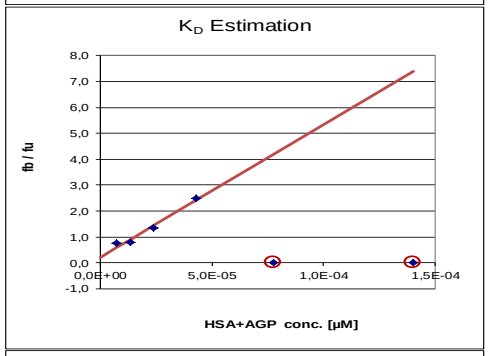
	-						
		missing values					
		1	1	1	1	0	0
		outlier detection					
consistency		ok	ok	ok	ok	**inconsistent**	**inconsistent**
saturation		ok	ok	ok	ok	**na**	**na**
statistics		ok	ok	ok	ok	**na**	**na**

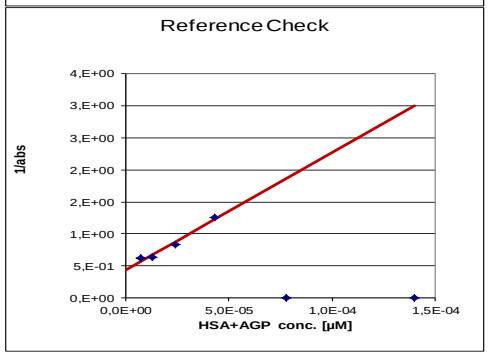
QC index: **7,4** **k_D= 1,95E-05** **fb=** **96,9% ± 0,332%** **r^2= 0,97262**

warning messages:

		consistency check					
signal < reference	4	1	1	1	1	0	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	4	1	1	1	1	0	0
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,25E-01	6,37E-01	8,33E-01	1,25E+00	#WERT!	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		42,9%	43,9%	57,1%	71,4%	0,0%	0,0%
(1-fu)/fu		0,750	0,783	1,333	2,500	0,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,750	0,783	1,333	2,500	0,000	0,000
slope		-9213,33988					
intercept		1,36394					
r^2		0,2457					
prediction: (1-fu)/fu		-1,1E+04	-1,1E+04	-1,1E+04	-1,1E+04	-1,1E+04	-1,1E+04
outlier due to saturation and consistency		1	1	1	1	0	0
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
(1-fu)/fu		0,750	0,783	1,333	2,500		
slope		51252,24619					
intercept		0,21465					
r^2		0,9726					
prediction		0,594	0,898	1,445	2,429		
residuals		0,156	-0,115	-0,112	0,071		
standarized residuals		1,151	0,000	0,000	0,523		
Threshold		1,5					
statistical outlier	4	1	1	1	1	0	0
		0,000	0,000	0,000	0,000		
		0,750	0,783	1,333	2,500		
slope		51252,24619	**1,95E-05**				
intercept		0,21465					
r^2		0,9726					
prediction	0,215	0,594	0,898	1,445	2,429	4,201	7,390
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	0,000	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,14	2,20	2,86	3,57	0,00	0,00
free protein con. [µM]		5,27	11,14	21,15	39,64	77,78	140,00
free protein con. [M]	0	5,3E-06	1,1E-05	2,1E-05	4,0E-05	7,8E-05	1,4E-04
binding sites covered [%]		28,9%	16,5%	11,9%	8,3%	0,0%	0,0%
warning?							







reg	**1,95E-05 ± 0,98**	**0,97262**	
avg	**1,32E-05 ± 0,98**		1
choice	**1,95E-05 ± 0,98**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		6,3E-01	6,4E-01	8,3E-01	1,3E+00	#WERT!	#WERT!
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
valid 1/signal		6,3E-01	6,4E-01	8,3E-01	1,3E+00		
slope		18304,37364					
intercept		0,43380					
r^2		0,9726					
predicted 1/signal	0	5,7E-01	6,8E-01	8,7E-01	1,2E+00	1,9E+00	3,0E+00
Predicted Reference Value		2,31					
Actual Reference Value		2,80					
Relative deviation		17,7%					

QC index: 7,4

no. data points	6	reference	9	r^2 MA predict	7	ref. Treatment	10	ref > signal	6	consistency	3					
		10,0%		0,9999		A	10	6		6						
6		20,0%	9	0,999		M		5		5						
5		50,0%	8	0,99		R		4	6	4	3					
4	6	100,0%	7	0,9	7	slopes	10	3		3						
3		200,0%	5	0,8	6	1/abs > 0	5	2		2						
2		500,0%	3	0,7	5	id)/c(buffer) >0	10	1		intercept						
1		<500%		0,6	4			0		0,21	10					
0				0,5	3											
				<0.5												

warning messages

data points	
reference	
ref. Treatment	
ref > signal references exceed	X
consistency data not consisten	X
r^2 MA prediction	
slopes	
intercept	
binding	
summary references exceed signal	
solubility problem?	data not consistent,

1. step outlier detection

mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	2,1E-10	7,5E-11	4,1E-12	4,5E-10		
$S(x_i\text{-mean}(x))^2$	7,4E-10					
	0,46	0,65	0,74	0,14		
residuals	0,156	-0,115	-0,112	0,071		
	0,0183					
	0,0121	0,0066	0,0062	0,0025		
s^2	0,0274					
studentized residual	1,38	-0,86	-0,78	1,13		
	1,38	0,86	0,78	1,13		
	1	1	1	1	0	0
	0,000	0,000	0,000	0,000		
	0,750	0,783	1,333	2,500		
slope	51252,24619					
intercept	0,21465					
r^2	0,9726					

initial r2: 0,9726

skip outlier detection if r2 > 0,98

consider outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	0	0
protein conc. [µM]	0,000	0,000	0,000	0,000		
(1-fu)/fu	0,8	0,783	1,333	2,500		
	51252,24619 w/o outlier		with outlier			
	0,21465	239,785	239,785	0,00 ok		
	0,9726 improvement?		no	1,0		
	accept outlier:		na			
abs(studentized residuals)	1,38	0,86	0,78	1,13		1,38
	7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05		
	0,8		1,3	2,5		

2. step outlier detection

mean x	0,000		n	4		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00		
$S(x_i\text{-mean}(x))^2$	0,00					
	0,46	0,65	0,74	0,14		
residuals	0,156	-0,115	-0,112	0,071		
	0,0121	0,0066	0,0062	0,0025		
s^2	0,0274					
studentized residual	1,38	-0,86	-0,78	1,13		
	1,38	0,86	0,78	1,13		
	1	1	1	1	0	0
	0,000	0,000	0,000	0,000		
	0,750	0,783	1,333	2,500		
slope	51252,24619	**1,95E-05**				
intercept	0,21465					
r^2	0,9726					

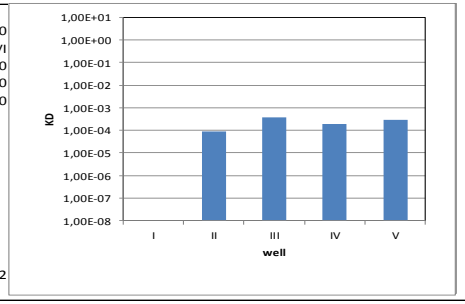
second tier r2: 0,9726

skip outlier detection if r2 > 0,98

consider outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	1	1	1	1	0	0
	I	II	III	IV	V	VI
[A] in [M]	2,857	2,804	2,143	1,429	5,000	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,143	2,196	2,857	3,571	0,000	0,000
KD	7,0E-06	1,4E-05	1,6E-05	1,6E-05		
	1,3E-05					
SE(KD)	2,108E-06					
fb	0,989	0,977	0,975	0,975		
	97,9%					
SE(fb)	0,332%					
n	4					
	7,02E-06	1,42E-05	1,59E-05	1,59E-05	1,00E-12	1,00E-12



test article No 1 **SL 13**

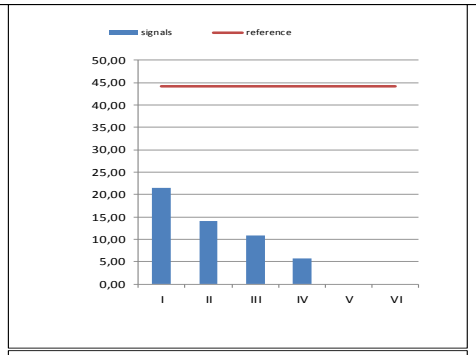
		5,00 µM				0 nm / amu	
adj. Ref 1		46,80	A		av ref ok		
Ref 2		41,70	ref diff ok		max ref ok		
Signal Reference:		44,25	44,25	44,25	44,25	44,25	44,25
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		21,40	14,00	10,90	5,70 na	na	ok

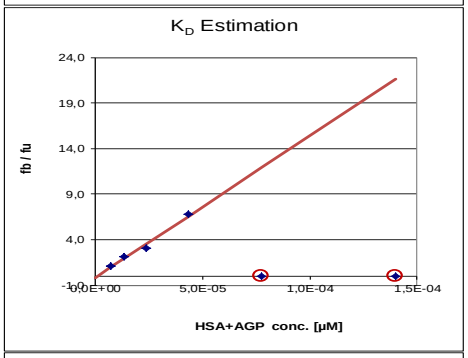
	-						
		missing values					
		1	1	1	1	0	0
		outlier detection					
consistency		ok	ok	ok	ok	**inconsistent**	**inconsistent**
saturation		ok	ok	ok	ok	**na**	**na**
statistics		ok	ok	ok	ok	**na**	**na**

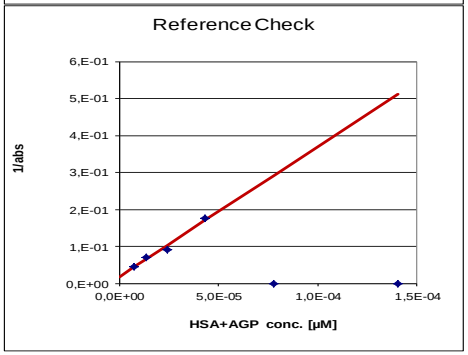
QC index: **7,4** **k_D= 6,43E-06** **fb= 99,0% ± 0,080%** **r^2= 0,97972**

warning messages: references exceed signals

		consistency check					
signal < reference	4	1	1	1	1	0	0
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	4	1	1	1	1	0	0
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		4,67E-02	7,14E-02	9,17E-02	1,75E-01	#WERT!	#WERT!
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		51,6%	68,4%	75,4%	87,1%	0,0%	0,0%
(1-fu)/fu		1,068	2,161	3,060	6,763	0,000	0,000
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	1
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		1,068	2,161	3,060	6,763	0,000	0,000
slope		-20605,08888					
intercept		3,22517					
r^2		0,1664					
prediction: (1-fu)/fu		-2,5E+04	-2,5E+04	-2,5E+04	-2,5E+04	-2,5E+04	-2,5E+04
outlier due to saturation and consistency		1	1	1	1	0	0
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
(1-fu)/fu		1,068	2,161	3,060	6,763		
slope		155574,63680					
intercept		-0,15830					
r^2		0,9797					
prediction		0,994	1,916	3,576	6,564		
residuals		0,073	0,244	-0,517	0,199		
standarized residuals		0,209	0,694	0,000	0,566		
Threshold		1,5					
statistical outlier	4	1	1	1	1	0	0
		0,000	0,000	0,000	0,000		
		1,068	2,161	3,060	6,763		
slope		155574,63680	**6,43E-06**				
intercept		-0,15830					
r^2		0,9797					
prediction	-0,158	0,994	1,916	3,576	6,564	11,942	21,622
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	0,000	0,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,58	3,42	3,77	4,36	0,00	0,00
free protein con. [µM]		4,83	9,92	20,24	38,85	77,78	140,00
free protein con. [M]	0	4,8E-06	9,9E-06	2,0E-05	3,9E-05	7,8E-05	1,4E-04
binding sites covered [%]		34,8%	25,6%	15,7%	10,1%	0,0%	0,0%
warning?							







reg	**6,43E-06 ± 0,99**	**0,97972**	
avg	**5,37E-06 ± 0,99**		1
choice	**6,43E-06 ± 0,99**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		4,7E-02	7,1E-02	9,2E-02	1,8E-01	#WERT!	#WERT!
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05		
valid 1/signal		4,7E-02	7,1E-02	9,2E-02	1,8E-01		
slope		3515,81100					
intercept		0,01902					
r^2		0,9797					
predicted 1/signal	0	4,5E-02	6,6E-02	1,0E-01	1,7E-01	2,9E-01	5,1E-01
Predicted Reference Value		52,57					
Actual Reference Value		44,25					
Relative deviation		15,8%					

QC index: 7,4

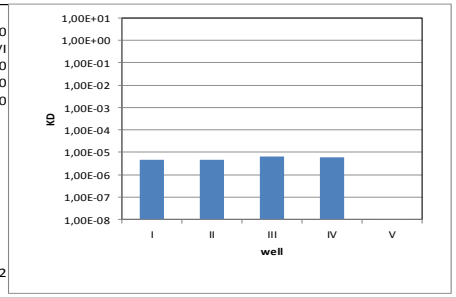
no. data points	6	reference	9	r^2 MA predict	7	ref. Treatment	10	ref > signal	6	consistency	3									
		10,0%		0,9999		A	10	6		6										
6		20,0%	9	0,999		M		5		5										
5		50,0%	8	0,99		R		4	6	4	3									
4	6	100,0%	7	0,9	7	slopes	10	3		3										
3		200,0%	5	0,8	6	1/abs > 0	5	2		2										
2		500,0%	3	0,7	5	c(lipid)/c(buffer) >0	10	1		intercept										
1		<500%		0,6	4			0		-0,16	10									
0				0,5	3															
				<0.5																

warning messages

data points	
reference	
ref. Treatment	
ref > signal	references exceed X
consistency	data not consisten X
r^2 MA prediction	
slopes	
intercept	
binding	
summary	references exceed signals
solubility problem?	data not consistent

		1. step outlier detection																		
mean x		0,000		n	4															
$(x_i\text{-mean}(x))^2$		2,1E-10	7,5E-11	4,1E-12	4,5E-10					initial r2:		0,9797								
$S(x_i\text{-mean}(x))^2$		7,4E-10								skip outlier detection if r2 >		0,98								
		0,46	0,65	0,74	0,14							consider	outlier detection							
residuals		0,073	0,244	-0,517	0,199															
		0,1239								check, if estimation of reference assay volume becomes worse after outlier detection										
		0,0027	0,0298	0,1335	0,0198															
s^2		0,1858										checking effect of outlier removal on intercept estimation of nt/c								
studentized residual		0,25	0,70	-1,39	1,22							1	1	1	1	0	0			
		0,25	0,70	1,39	1,22					protein conc. [µM]		0,000	0,000	0,000	0,000					
		1	1	1	1	0	0			(1-fu)/fu		1,1	2,161	3,060	6,763					
		0,000	0,000	0,000	0,000							155574,63680	w/o outlier	with outlier						
		1,068	2,161	3,060	6,763							-0,15830	240,158	240,158	0,00	ok				
slope		155574,63680										0,9797	improvement?	no	1,0					
intercept		-0,15830											accept outlier:	**na**						
r^2		0,9797								abs(studentized residuals)		0,25	0,70	1,39	1,22					1,39
		2. step outlier detection										7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05					
mean x		0,000		n	4							1,1	2,2		6,8					
$(x_i\text{-mean}(x))^2$		0,00	0,00	0,00	0,00															
$S(x_i\text{-mean}(x))^2$		0,00																		
		0,46	0,65	0,74	0,14															
residuals		0,073	0,244	-0,517	0,199					second tier r2:		0,9797								
		0,0027	0,0298	0,1335	0,0198					skip outlier detection if r2 >		0,98								
s^2		0,1858										consider	outlier detection							
studentized residual		0,25	0,70	-1,39	1,22															
		0,25	0,70	1,39	1,22					no outliers will be recorded if there are only 3 or less points left at step 1 or 2										
		1	1	1	1	0	0													
		0,000	0,000	0,000	0,000															
		1,068	2,161	3,060	6,763															
slope		155574,63680	**6,43E-06**																	
intercept		-0,15830																		
r^2		0,9797																		

	Standard Error					
	1	1	1	1	0	0
	I	II	III	IV	V	VI
[A] in [M]	2,418	1,582	1,232	0,644	5,000	5,000
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,582	3,418	3,768	4,356	0,000	0,000
KD	4,5E-06	4,6E-06	6,6E-06	5,7E-06		
	5,4E-06					
SE(KD)	5,015E-07					
fb	0,993	0,993	0,989	0,991		
	99,1%					
SE(fb)	0,080%					
n	4					
	4,52E-06	4,59E-06	6,61E-06	5,74E-06	1,00E-12	1,00E-12



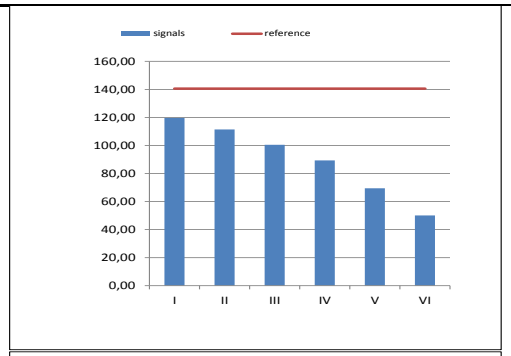
test article No 2 **SL 11**

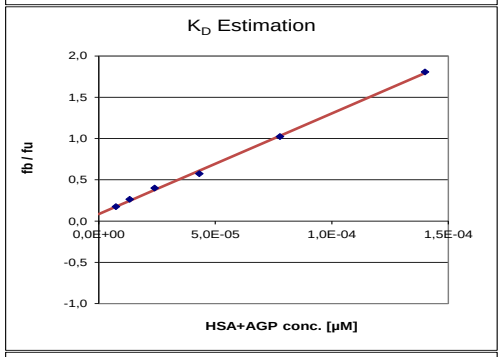
		5,00 µM				0 nm / amu	
adj. Ref 1		145,00	A		av ref ok		
Ref 2		136,20	ref diff ok		max ref ok		
Signal Reference:		140,60	140,60	140,60	140,60	140,60	140,60
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		119,70	111,40	100,50	89,40	69,50	50,10	ok
		-					
		missing values					
		1	1	1	1	1	1
		outlier detection					
consistency		ok	ok	ok	ok	ok	ok
saturation		ok	ok	ok	ok	ok	ok
statistics		ok	ok	ok	ok	ok	ok

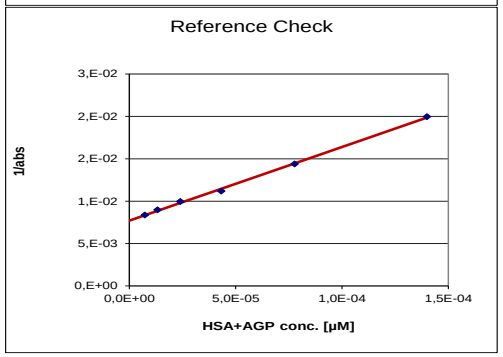
QC index: 9,7 **k_D= 8,19E-05** **fb= 88,2% ± 0,876%** **r^2= 0,99868**

warning messages:

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	6	1	1	1	1	1	1
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		8,35E-03	8,98E-03	9,95E-03	1,12E-02	1,44E-02	2,00E-02
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		14,9%	20,8%	28,5%	36,4%	50,6%	64,4%
(1-fu)/fu		0,175	0,262	0,399	0,573	1,023	1,806
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,175	0,262	0,399	0,573	1,023	1,806
slope		12210,08458					
intercept		0,08412					
r^2		0,9987					
prediction: (1-fu)/fu		1,5E+04	1,5E+04	1,5E+04	1,5E+04	1,5E+04	1,5E+04
outlier due to saturation and consistency		1	1	1	1	1	1
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	0,000
(1-fu)/fu		0,175	0,262	0,399	0,573	1,023	1,806
slope		12210,08458					
intercept		0,08412					
r^2		0,9987					
prediction		0,175	0,247	0,377	0,612	1,034	1,794
residuals		0,000	0,015	0,022	-0,039	-0,011	0,013
standarized residuals		0,001	0,677	0,972	0,000	0,000	0,574
Threshold		1,5					
statistical outlier	6	1	1	1	1	1	1
		0,000	0,000	0,000	0,000	0,000	0,000
		0,175	0,262	0,399	0,573	1,023	1,806
slope		12210,08458	**8,19E-05**				
intercept		0,08412					
r^2		0,9987					
prediction	0,084	0,175	0,247	0,377	0,612	1,034	1,794
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	-5,000
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		0,74	1,04	1,43	1,82	2,53	3,22
free protein con. [µM]		6,67	12,30	22,58	41,39	75,25	136,78
free protein con. [M]	0	6,7E-06	1,2E-05	2,3E-05	4,1E-05	7,5E-05	1,4E-04
binding sites covered [%]		10,0%	7,8%	5,9%	4,2%	3,3%	2,3%
warning?							







reg	**8,19E-05 ± 0,91**	**0,99868**	
avg	**6,05E-05 ± 0,91**		1
choice	**8,19E-05 ± 0,91**		

value of "-5.0" places outlier markers below visible chart area

		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		8,4E-03	9,0E-03	1,0E-02	1,1E-02	1,4E-02	2,0E-02
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
valid 1/signal		8,4E-03	9,0E-03	1,0E-02	1,1E-02	1,4E-02	2,0E-02
slope		86,84271					
intercept		0,00771					
r^2		0,9987					
predicted 1/signal	0	8,4E-03	8,9E-03	9,8E-03	1,1E-02	1,4E-02	2,0E-02
Predicted Reference Value		129,69					
Actual Reference Value		140,60					
Relative deviation		7,8%					

QC index: 9,7

													warning messages
no. data points	10	reference	10	r^2 MA predicl	8	ref. Treatment	10	ref > signal	10	consistency	10		data points
		10,0%	10	0,9999		A	10	6	10	6	10		reference
6	10	20,0%	9	0,999		M		5		5			ref. Treatment
5		50,0%	8	0,99	8	R		4		4			ref > signal
4		100,0%	7	0,9	7	slopes	10	3		3			consistency
3		200,0%	5	0,8	6	1/abs > 0	5	2		2			r^2 MA prediction
2		500,0%	3	0,7	5	id)/c(buffer) >0	10	1			intercept		slopes
1		<500%		0,6	4			0		0,08	10		intercept
0				0,5	3								binding
				<0.5									summary

	1. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	1,9E-09	1,4E-09	7,3E-10	6,0E-11	7,2E-10	7,9E-09
$S(x_i\text{-mean}(x))^2$	1,3E-08					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,000	0,015	0,022	-0,039	-0,011	0,013
	0,0005					
	0,0000	0,0001	0,0001	0,0004	0,0000	0,0000
s^2	0,0006					
studentized residual	0,00	0,71	0,99	-1,71	-0,49	1,12
	0,00	0,71	0,99	1,71	0,49	1,12
	1	1	1	1	1	1
	0,000	0,000	0,000	0,000	0,000	0,000
	0,175	0,262	0,399	0,573	1,023	1,806
slope	12210,08458					
intercept	0,08412					
r^2	0,9987					

	2. step outlier detection					
mean x	0,000		n	6		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00	0,00
$S(x_i\text{-mean}(x))^2$	0,00					
	0,68	0,72	0,78	0,83	0,78	0,21
residuals	0,000	0,015	0,022	-0,039	-0,011	0,013
	0,0000	0,0001	0,0001	0,0004	0,0000	0,0000
s^2	0,0006					
studentized residual	0,00	0,71	0,99	-1,71	-0,49	1,12
	0,00	0,71	0,99	1,71	0,49	1,12
	1	1	1	1	1	1
	0,000	0,000	0,000	0,000	0,000	0,000
	0,175	0,262	0,399	0,573	1,023	1,806
slope	12210,08458	**8,19E-05**				
intercept	0,08412					
r^2	0,9987					

initial r2:	0,9987	
skip outlier detection if r2 >	0,98	
	omit	outlier detection

check, if estimation of reference assay volume becomes worse after outlier detection

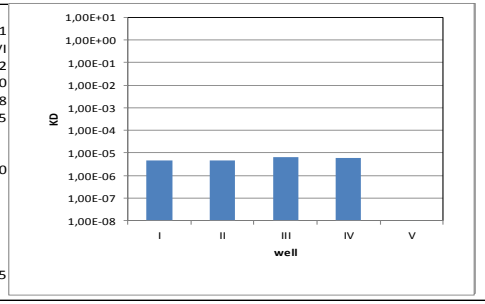
checking effect of outlier removal on intercept estimation of nt/c

	1	1	1	1	1	1
protein conc. [µM]	0,000	0,000	0,000	0,000	0,000	0,000
(1-fu)/fu	0,2	0,262	0,399	0,573	1,023	1,806
	12210,08458 w/o outlier		with outlier			
	0,09564	239,904	239,916	0,00 ok		
	0,9987 improvement?		yes	1,0		
	accept outlier:		**na**			
abs(studentized residuals)	0,00	0,71	0,99	1,71	0,49	1,12
	7,4091E-06	1,33364E-05	2,40055E-05		7,77778E-05	0,00014
	0,2	0,3	0,4		1,0	1,8

second tier r2:	0,9987	
skip outlier detection if r2 >	0,98	
	omit	outlier detection

no outliers will be recorded if there are only 3 or less points left at step 1 or 2

	Standard Error					
	1	1	1	1	1	1
	I	II	III	IV	V	VI
[A] in [M]	4,257	3,962	3,574	3,179	2,472	1,782
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	0,743	1,038	1,426	1,821	2,528	3,218
KD	3,8E-05	4,7E-05	5,7E-05	7,2E-05	7,4E-05	7,6E-05
	6,1E-05					
SE(KD)	6,426E-06					
fb	0,941	0,929	0,915	0,894	0,893	0,890
	91,0%					
SE(fb)	0,876%					
n	6					
	3,82E-05	4,69E-05	5,66E-05	7,23E-05	7,36E-05	7,57E-05



test article No 3 **SL 10**

		5,00 µM				0 nm / amu	
adj. Ref 1		11,10	A		av ref ok		
Ref 2		11,10	ref diff ok		max ref ok		
Signal Reference:		11,10	11,10	11,10	11,10	11,10	11,10
TRANSIL well:		I	II	III	IV	V	VI
Signal in TRANSIL wells:		5,80	3,70	2,60	2,10	1,20	2,50 ok

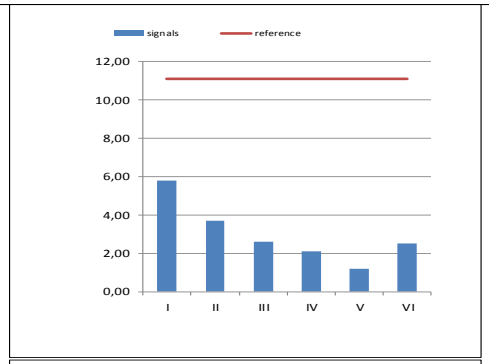
	missing values					
	1	1	1	1	1	1
	outlier detection					
consistency	ok	ok	ok	ok	ok	**inconsistent**
saturation	ok	ok	ok	ok	ok	**na**
statistics	ok	ok	ok	ok	ok	**na**

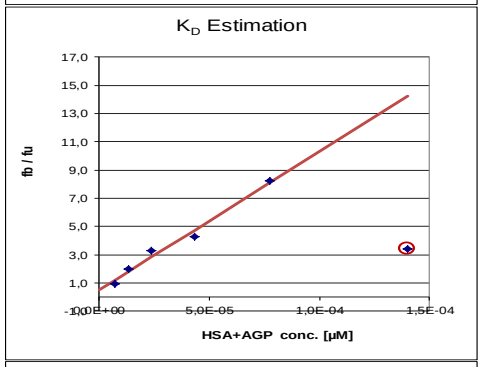
r^2= **0,98352**

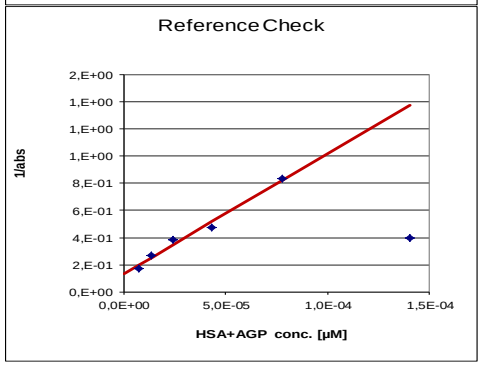
QC index: 8,6 **k_D= 1,02E-05** **fb= 98,4% ± 0,138%**

warning messages: data not consistent,

		consistency check					
signal < reference	6	1	1	1	1	1	1
binding > 0%		1	1	1	1	1	1
steady increase in binding & summary	5	1	1	1	1	1	0
		removal of outliers due to saturation					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,72E-01	2,70E-01	3,85E-01	4,76E-01	8,33E-01	4,00E-01
nt [nmol]		1,2	1,2	1,2	1,2	1,2	1,2
fb in assay		47,7%	66,7%	76,6%	81,1%	89,2%	77,5%
(1-fu)/fu		0,914	2,000	3,269	4,286	8,250	3,440
saturation threshold		99,999%					
systematic outlier (saturation)		1	1	1	1	1	0
consistent saturation		1	1	1	1	1	1
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		0,914	2,000	3,269	4,286	8,250	3,440
slope		22849,58982					
intercept		2,52879					
r^2		0,2083					
prediction: (1-fu)/fu		2,7E+04	2,7E+04	2,7E+04	2,7E+04	2,7E+04	2,7E+04
outlier due to saturation and consistency		1	1	1	1	1	0
		statistical outlier detection & removal					
protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
(1-fu)/fu		0,914	2,000	3,269	4,286	8,250	
slope		98492,21374					
intercept		0,47895					
r^2		0,9835					
prediction		1,209	1,792	2,843	4,735	8,139	
residuals		-0,295	0,208	0,426	-0,449	0,111	
standarized residuals		0,000	0,573	1,175	0,000	0,305	
Threshold		1,5					
statistical outlier	5	1	1	1	1	1	0
		0,000	0,000	0,000	0,000	0,000	
		0,914	2,000	3,269	4,286	8,250	
slope		98492,21374	**1,02E-05**				
intercept		0,47895					
r^2		0,9835					
prediction	0,479	1,209	1,792	2,843	4,735	8,139	14,268
		outlier					
effective protein conc. [M]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
(1-fu)/fu		-5,000	-5,000	-5,000	-5,000	-5,000	3,440
		Binding QC					
no. of binding sites		1					
conc. protein-compound complex [µM]		2,39	3,33	3,83	4,05	4,46	3,87
free protein con. [µM]		5,02	10,00	20,18	39,16	73,32	136,13
free protein con. [M]	0	5,0E-06	1,0E-05	2,0E-05	3,9E-05	7,3E-05	1,4E-04
binding sites covered [%]		32,2%	25,0%	15,9%	9,4%	5,7%	2,8%
warning?							







reg	**1,02E-05 ± 0,99**	**0,98352**	
avg	**6,94E-06 ± 0,99**		1
choice	**1,02E-05 ± 0,99**		

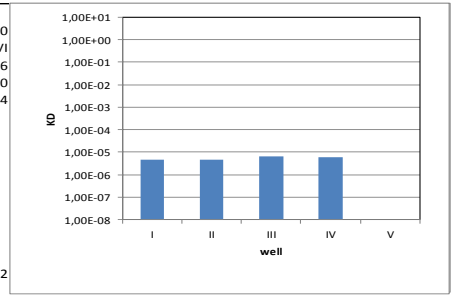
value of "-5.0" places outlier markers below visible chart area

		reference QC					
protein conc. [M]	0	7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	1,4E-04
1/signal in TRANSIL well		1,7E-01	2,7E-01	3,8E-01	4,8E-01	8,3E-01	4,0E-01
valid protein conc. [µM]		7,4E-06	1,3E-05	2,4E-05	4,3E-05	7,8E-05	
valid 1/signal		1,7E-01	2,7E-01	3,8E-01	4,8E-01	8,3E-01	
slope		8873,17241					
intercept		0,13324					
r^2		0,9835					
predicted 1/signal	0	2,0E-01	2,5E-01	3,5E-01	5,2E-01	8,2E-01	1,4E+00
Predicted Reference Value		7,51					
Actual Reference Value		11,10					
Relative deviation		32,4%					

				QC index: 8,6												warning messages		
no. data points	9	reference	8	r^2 MA predicl	7	ref. Treatment	10	ref > signal	10	consistency	6					data points		
		10,0%		0,9999		A	10	6	10	6						reference		
6		20,0%		0,999		M		5		5	6					ref. Treatment		
5	9	50,0%	8	0,99		R		4		4						ref > signal		
4		100,0%	7	0,9	7	slopes	10	3		3						consistency data not consisten	X	
3		200,0%	5	0,8	6	1/abs > 0	5	2		2						r^2 MA prediction		
2		500,0%	3	0,7	5	1)/c(buffer) >0	10	1		intercept						slopes		
1		<500%		0,6	4			0		0,48	10					intercept		
0				0,5	3											binding		
				<0.5												summary data not consistent,		

	1. step outlier detection														
mean x	0,000		n	5											
$(x_i\text{-mean}(x))^2$	6,6E-10	3,9E-10	8,4E-11	1,0E-10	2,0E-09			initial r2:	0,9835						
$S(x_i\text{-mean}(x))^2$	3,2E-09							skip outlier detection if r2 >	0,98						
	0,60	0,68	0,77	0,77	0,18				omit	outlier detection					
residuals	-0,295	0,208	0,426	-0,449	0,111										
	0,1313							check, if estimation of reference assay volume becomes worse after outlier detection							
	0,0290	0,0144	0,0605	0,0672	0,0041										
s^2	0,1751								checking effect of outlier removal on intercept estimation of nt/c						
studentized residual	-0,91	0,60	1,16	-1,22	0,62				1	1	1	1	1	0	
	0,91	0,60	1,16	1,22	0,62			protein conc. [µM]	0,000	0,000	0,000	0,000	0,000		
	1	1	1	1	1	0		(1-fu)/fu	0,9	2,000	3,269	4,286	8,250		
	0,000	0,000	0,000	0,000	0,000				98492,21374	w/o outlier	with outlier				
	0,914	2,000	3,269	4,286	8,250				0,47895	239,521	239,521	0,00	ok		
slope	98492,21374								0,9835	improvement?	no	1,0			
intercept	0,47895								accept outlier:	**na**					
r^2	0,9835							abs(studentized residuals)	0,91	0,60	1,16	1,22	0,62		1,22
	2. step outlier detection								7,4091E-06	1,33364E-05	2,40055E-05	4,32099E-05	7,77778E-05		
mean x	0,000		n	5					0,9	2,0	3,3		8,3		
$(x_i\text{-mean}(x))^2$	0,00	0,00	0,00	0,00	0,00										
$S(x_i\text{-mean}(x))^2$	0,00														
	0,60	0,68	0,77	0,77	0,18										
residuals	-0,295	0,208	0,426	-0,449	0,111			second tier r2:	0,9835						
	0,0290	0,0144	0,0605	0,0672	0,0041			skip outlier detection if r2 >	0,98						
s^2	0,1751								omit	outlier detection					
studentized residual	-0,91	0,60	1,16	-1,22	0,62										
	0,91	0,60	1,16	1,22	0,62			no outliers will be recorded if there are only 3 or less points left at step 1 or 2							
	1	1	1	1	1	0									
	0,000	0,000	0,000	0,000	0,000										
	0,914	2,000	3,269	4,286	8,250										
slope	98492,21374	**1,02E-05**													
intercept	0,47895														
r^2	0,9835														

	Standard Error					
	1	1	1	1	1	0
	I	II	III	IV	V	VI
[A] in [M]	2,613	1,667	1,171	0,946	0,541	1,126
[B] in [M]	7,409	13,336	24,005	43,210	77,778	140,000
[AB] in [M]	2,387	3,333	3,829	4,054	4,459	3,874
KD	5,5E-06	5,0E-06	6,2E-06	9,1E-06	8,9E-06	
	6,9E-06					
SE(KD)	8,675E-07					
fb	0,991	0,992	0,990	0,985	0,986	
	98,9%					
SE(fb)	0,138%					
n	5					
	5,50E-06	5,00E-06	6,17E-06	9,14E-06	8,89E-06	1,00E-12



4.5. Chromatogramme

Als Beispiel für eine Chromatogramm-Serie von HSA, AGP und PPB wurde die Probe SL 6 gewählt. Die Abnahme der Proteinbindung von Well 1 bis Well 6 ist gut erkennbar.

4.5.1. HSA-Chromatogramme

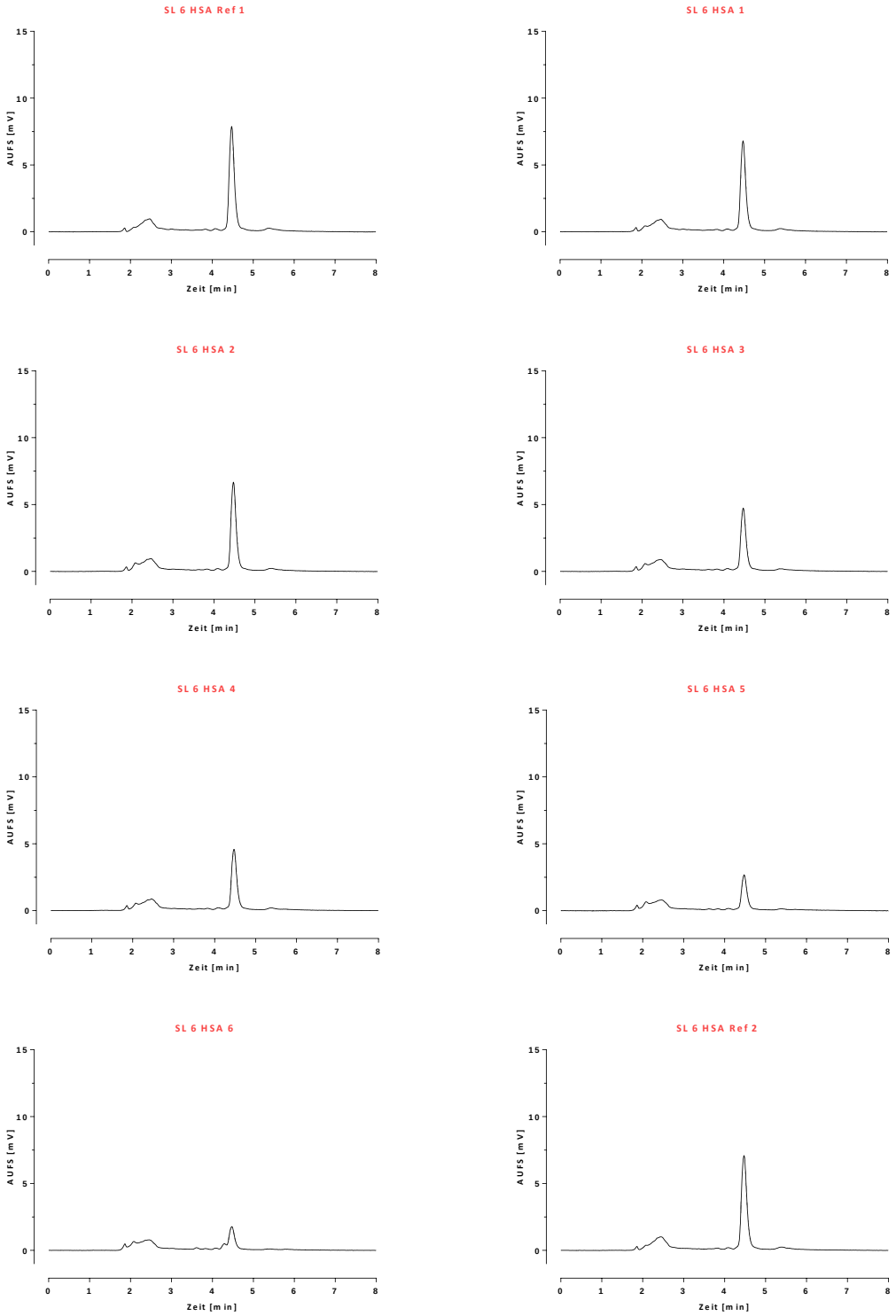


Abb. 22: Chromatogramm-Serie HSA (GraphPad Prism Screenshot)

4.5.2. AGP-Chromatogramme

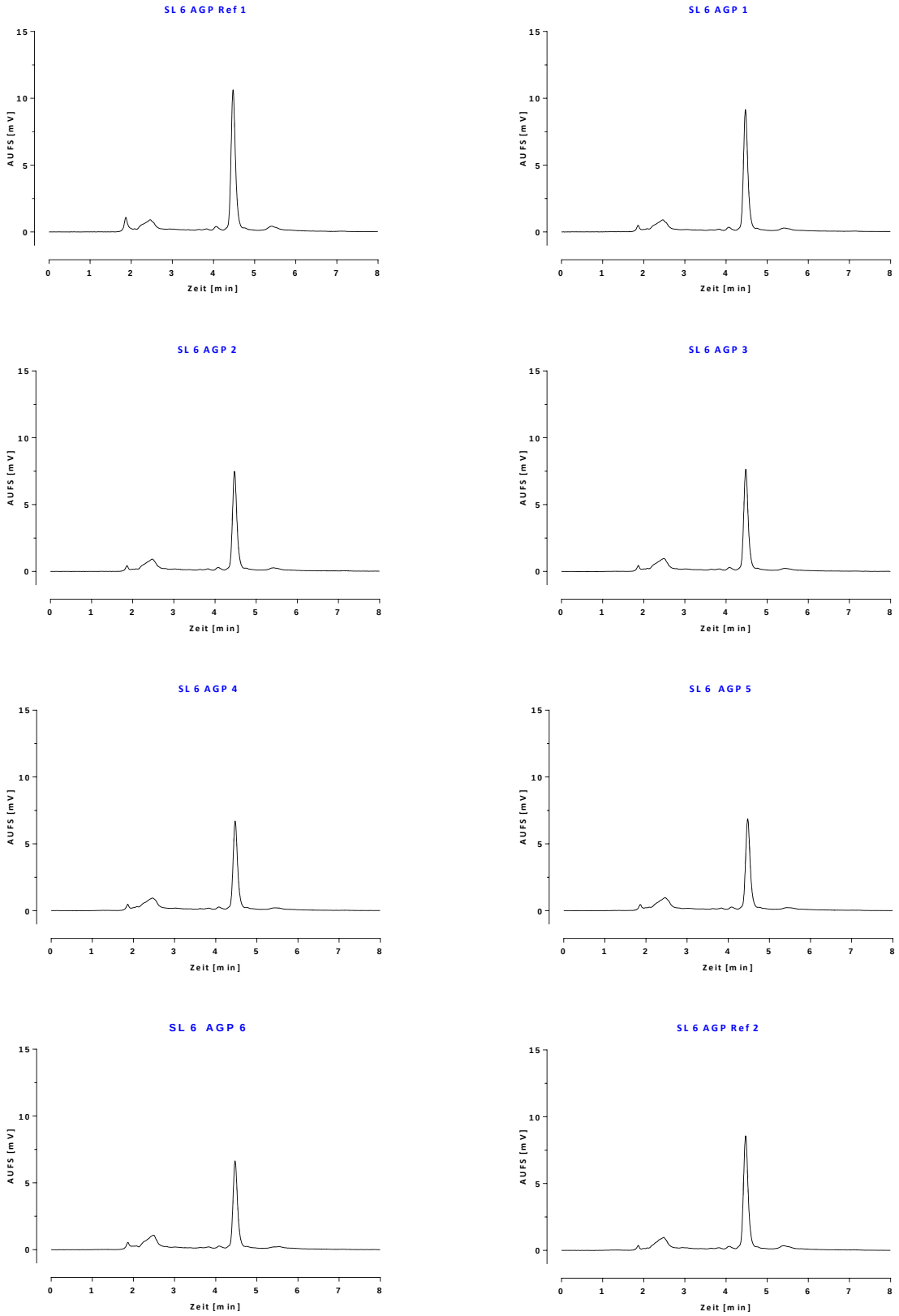


Abb. 23: Chromatogramm-Serie AGP (GraphPad Prism Screenshot)

4.5.3. PPB-Chromatogramme

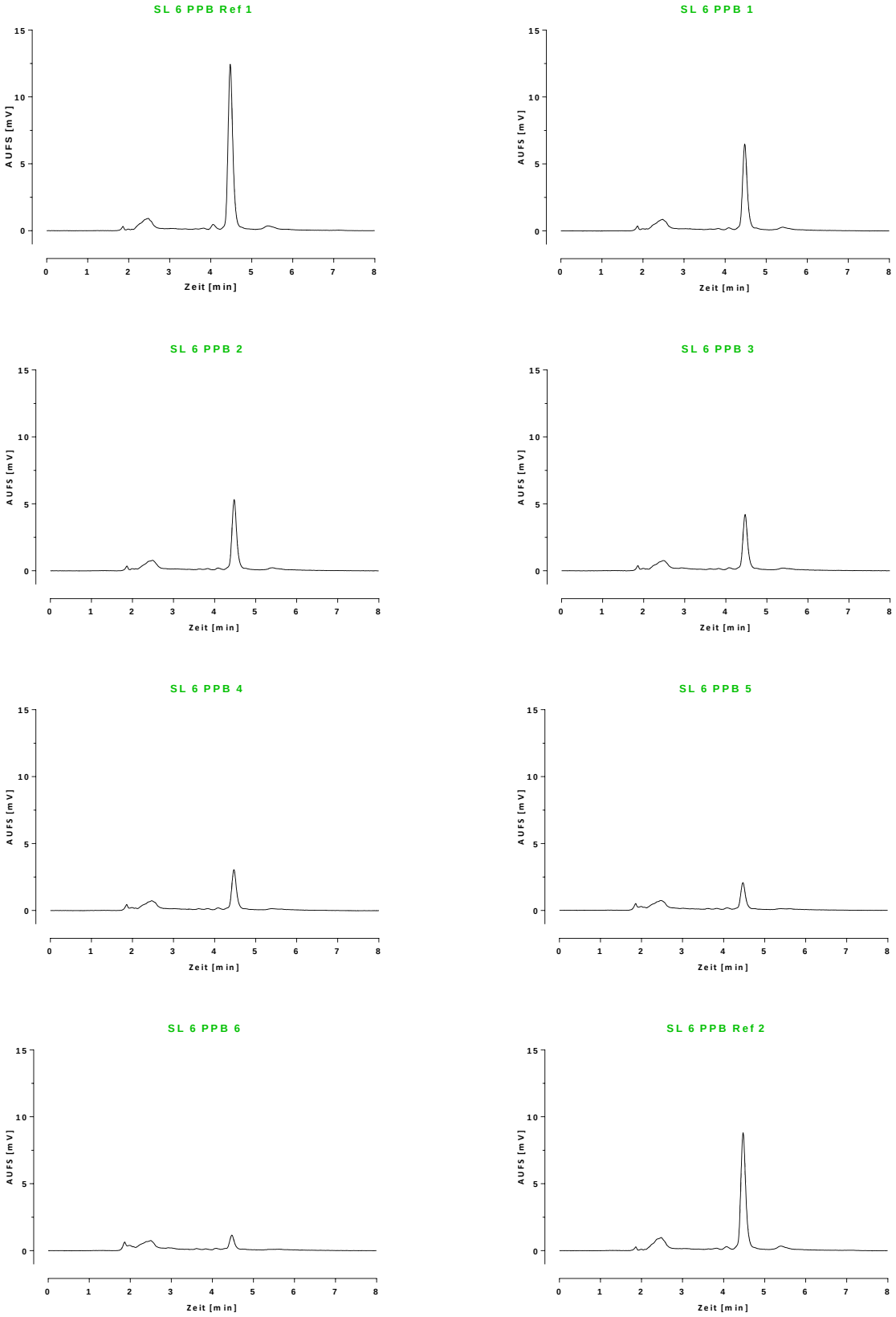


Abb. 24: Chromatogramm-Serie PPB (GraphPad Prism Screenshot)

4.6. Andere verwendete Software

- Microsoft Word 2010: zum Erstellen der Diplomarbeit
- Microsoft Excel 2010: zur Auswertung der HPLC Analyse der Transil^{XL} Kits
- GraphPad Prism 6.0: zum Erstellen der Chromatogramm-Diagrammserie der HPLC Auswertung
- MS Paint 2010: zum Erstellen von Screenshots für Abbildungen

5. Zusammenfassung

In der vorliegenden Diplomarbeit wurden präklinische Untersuchungen zur Plasmaproteinbindung mit neuen Anthrachinon-Derivaten antibiotischer Aktivität durchgeführt. Als positiver Standard und zur Kontrolle wurde auch die Bindung des bereits etablierten Arzneistoffes Doxorubin.HCl getestet.

Die Bindung der SL-Substanzen SL1 bis SL 14 der Firma Sea-Life Pharma GmbH und Doxorubicin.HCl wurde mit den in Vitro Testsystemen „Transil^{XL}- Binding Kits“ der Firma Sovicell an die Proteine Human Serum Albumin (HSA) und alpha 1-azides Glykoprotein (AGP) untersucht. Dabei wurden sowohl der freie und gebundene Anteil an HSA und AGP, als auch die Proteinbindung an beide Proteine gleichzeitig ermittelt. Die Quantifizierung der Proben erfolgte durch eine HPLC-Analyse. Durch die Peakflächen und eine zu den Transil^{XL}-Testkits gehörige Microsoft Excel-Software war eine genaue und effiziente Auswertung möglich.

%	SL 1	SL 2	SL 3	SL 4	SL 5	SL 6	SL 7	SL 8	SL 9	SL 10	SL 11	SL 12	SL 13	SL 14	Doxo. HCl
HSA fb	92,8	93,5	90,0	98,5	-	93,3	94,2	94,9	88,8	97,5	90,4	98,5	99,8	94,9	61,2
HSA fu	7,2	6,4	9,3	<1,5	-	6,7	3	4,2	6,9	2,2	9,4	1,5	0,2	3,9	38,1
TQI	8,1	9,6	7,2	7,4	-	9,4	9,6	8,2	9,5	6,9	8,2	7,1	7,4	8,1	9,4
AGP fb	7,2	21	44,4	<1,4	<1,4	11,1	94,3	79,8	84,6	83,1	18,6	44,9	38,8	86,2	4,7
AGP fu	7,2	6,4	9,3	1,5	-	6,7	3,0	4,2	6,9	2,2	9,4	1,5	0,2	3,9	38,1
TQI	7,9	7,8	8,1	2,8	4,6	8,8	6,4	9,4	8,4	8,0	6,7	9,6	8,4	7,3	8,8
PPB fb	93,9	96,4	96,9	99,1	91	95,8	97,7	98,9	94,6	98,4	88,2	99,1	99,0	97,2	66,3
TQI	9,6	9,5	7,4	7,6	6,1	8,6	7,6	9,6	9,6	8,6	9,7	8,3	7,4	9,6	9,3

Tab. 8: Proteinbindungs-Ergebnisse

Laut den Ergebnissen aus Tabelle 8 ist zu entnehmen, dass die SL-Substanzen eine durchwegs sehr hohe Proteinbindung an HSA und AGP gleichzeitg (PPB fb) aufweisen.

Die Bindung an HSA zeichnet sich ebenfalls durch eine hohe Bindungsrate aus. Die fraction unbound von HSA liegt in jedem Fall unter 10%.

Der gebundene Anteil an AGP der Proben-Substanzen reicht von <1,4% bis 94,3%. Der ungebundene Anteil liegt auch hier überall unter 10%.

Durch den patentrechtlichen Schutz ist leider keine exakte Aussage über das Bindungsprofil der SL-Substanzen möglich, da keine Stellungnahme zu den unterschiedlichen Resten, in denen sich die Probe unterscheiden, gemacht werden kann.

Die Plasmaprotein-Bindung von Doxorubicin-HCl stimmt mit der Literatur überein. [23]

Der Transil Quality Index, TQI, gibt die Güte der Messungen an. Hier ist ersichtlich, dass sich der TQI der Substanzen, bis auf SL 5 für HSA und AGP; und SL 4 für AGP, im oberen Bereich von 6 bis 10 aufhält.

SL 4 war die unbeständigste Substanz, ohne Lichtschutz wäre hier gar keine Quantifizierung möglich gewesen.

SL 5 war trotz seiner guten Löslichkeit (Tab. 5) kaum ansprechbar auf das Test Kit, viele Peakflächen seiner Chromatogramme aus der HSA und PPB Testung waren nicht messbar. (s. 12.1.).

Die Arbeit mit den Binding-Kits war unkompliziert und äußerst effizient. Einzig die richtige Konzentration der Stammlösung herzustellen nahm anfangs etwas länger in Anspruch.

Wichtig zu beachten ist, dass die Transil^{XL} Technologie auf gereinigten Proteinen basiert, alle binding sites der Moleküle sind dabei gänzlich verfügbar. Dieser Zustand muss nicht für die Bedingungen der Protein-Bindung im Körper gelten. Da nur AGP und HSA, und im PPB-Binding Kit beide kombiniert, berücksichtigt wurden, kann es zu einer Unterschätzung der Proteinbindung kommen, da die Bindung an Lipoproteine oder andere Plasmaproteine in geringerer Konzentration nicht beachtet wird.

Jedenfalls stellen die Ergebnisse der vorliegenden Diplomarbeit wertvolle Erkenntnisse zur Abschätzung eines intravasalen Depots für SL-compounds dar. Damit können, nach Vergleich mit in-vivo Daten, pharmakokinetische Überlegungen zur Dosierung etc. getroffen werden.

6. Summary

In the present diploma thesis preclinical studies were conducted to plasma protein binding with new anthraquinone derivatives of antibiotic activity. As positive reference the plasma protein binding of the already established drug Doxorubin.HCl was tested. The protein binding of the SL-substances SL1-14, which were synthesized by SeaLife Pharma GmbH Company and Doxorubicin.HCl was explored with in Vitro Transil^{XL} binding testkits by Sovicell GmbH Company, to the proteins human serum albumin (HSA) and alpha 1-acid glycoprotein (AGP). Both fraction bound and unbound were tested with the HSA and AGP binding kits. The third binding kit, PPB, allowed a simultanenous testing to HSA and AGP.

The quantification of the samples was performed by HPLC analysis. With the peak areas from the HPLC analysis and the Microsoft Excel software a simple and efficient evaluation was possible.

%	SL 1	SL 2	SL 3	SL 4	SL 5	SL 6	SL 7	SL 8	SL 9	SL 10	SL 11	SL 12	SL 13	SL 14	Doxo. HCl
HSA fb	92,8	93,5	90,0	98,5	-	93,3	94,2	94,9	88,8	97,5	90,4	98,5	99,8	94,9	61,2
HSA fu	7,2	6,4	9,3	<1,5	-	6,7	3	4,2	6,9	2,2	9,4	1,5	0,2	3,9	38,1
TQI	8,1	9,6	7,2	7,4	-	9,4	9,6	8,2	9,5	6,9	8,2	7,1	7,4	8,1	9,4
AGP fb	7,2	21	44,4	<1,4	<1,4	11,1	94,3	79,8	84,6	83,1	18,6	44,9	38,8	86,2	4,7
AGP fu	7,2	6,4	9,3	1,5	-	6,7	3,0	4,2	6,9	2,2	9,4	1,5	0,2	3,9	38,1
TQI	7,9	7,8	8,1	2,8	4,6	8,8	6,4	9,4	8,4	8,0	6,7	9,6	8,4	7,3	8,8
PPB fb	93,9	96,4	96,9	99,1	91	95,8	97,7	98,9	94,6	98,4	88,2	99,1	99,0	97,2	66,3
TQI	9,6	9,5	7,4	7,6	6,1	8,6	7,6	9,6	9,6	8,6	9,7	8,3	7,4	9,6	9,3

Tab. 9: protein binding results

According to the results from table 8 it can be seen that the SL substances have a consistently very high protein binding to HSA and AGP simultaneously (PPB fb).

The binding to HSA is also characterized by a high binding rate. The unbound fraction of HSA is in any case less than 10%.

The fraction bound of the AGP sample substances is ranging from <1.4% to 94.3%. The unbound fraction is also lower than 10%.

Due to the patent protection no exact information on the binding profile of the SL substances is possible, because no statement about the functional groups, in which the samples differ, can be made.

The PPB of Doxorubicin.HCl is consistent with the literature. [23]

The Transil Quality Index, TQI, indicates the quality of the measurements. The chart shows that the TQI of the substances, except for SL 5 for HSA and AGP; and for SL 4 for AGP, resides in the upper range of 6 to 10.

SL 4 was the most volatile substance, without light shield no quantification would have been possible.

SL 5 was in spite of its good solubility (s. Table 5) hardly responsive to the test kit; many of its peak areas from the chromatograms series of HSA and PPB testing were not measurable. (s.a.12.1.)

Working with the binding kits was straightforward and extremely efficient. Only the preparation of the stock solution's correct concentration took a little longer in demanding.

It is important to consider that Transil^{XL} technology is based on purified proteins, therefore, all binding sites are expected to be available. In human plasma the binding sites may be masked, this may result in higher binding than estimated by assay.

Since only the measurement of AGP and HSA binding, and in the PPB-Binding Kit both combined, were taken into account, it may lead to an underestimation of protein binding, because the binding to lipoproteins or other plasma proteins of lower abundance is not observed.

In any case, the results of the present diploma thesis show a valuable knowledge for the evaluation of an intravascular deposit for SL-compounds. After a comparison with, for example, in-vivo data, pharmacokinetic considerations to the dosage etc. can be met.

7. Conclusio

Die vorliegenden Ergebnisse in dieser Diplomarbeit zeigen die Bindung von neuen Arzneistoffkandidaten an die Plasmaproteine HSA und AGP. Durch den etablierten Arzneistoff Doxorubicin.HCl als positiven Standard wurde die Reproduzierbarkeit der Testsysteme bestätigt.

Durch die sehr hohe Proteinbindung könnte es in dieser Substanzklasse allerdings zu Problemen in der Dosierung kommen. Um die MIC (minimal inhibitoric concentration), die sowohl von der Substanz, als auch von der Ansprechbarkeit und der Sensitivität des zu bekämpfenden Keims abhängt, zu erreichen, könnten sehr hohe Dosen von Nöten sein. In der Arbeit meiner Kollegin[1] wurde neben der mikrosomalen und intestinalen Bindung, mit den K_D-Werten aus meiner Diplomarbeit, auch das scheinbare Verteilungsvolumen V_D errechnet, mit dem eine genauere Aussage zur Distribution gemacht werden kann.

Für weitere Aussagen bezüglich anderer Parameter aus dem ADME-Modell, sind auf jeden Fall weitere Untersuchungen notwendig, um die SL-Substanzen auf vielversprechende Arzneistoffkandidaten einzugrenzen und weiter zu untersuchen.

[1] Valerie Klug: Untersuchung zur Gewebsaufnahme und Gewebsbindung ausgewählter Anthrachinone, 2014

8. Abkürzungsverzeichnis

AAG	α_1-azides Glykoprotein
ACN	Acetonitril
AGP	α_1-azides Glykoprotein
ATP	Adenosin-Tri-Phosphat
AUFS	area under full scale
DMSO	Dimethylsulfoxid
Doxo.HCl	Doxorubicin.HCl
Eprouv.	Eprouvette
EW	Einwaage
f_b	fraction bound, gebundener Anteil
f_u	fraction unbound, freier Anteil
HPLC	High-performance liquid chromatography
HSA	Human Serum Albumin
IL-1/ IL-6	Interleukin-1/ Interleukin-6
K_D	Dissoziationskonstante K, auch: Affinitätskonstante K
kDa	kilo Dalton
ADME	Absorption, Distribution, Metabolismus, Exkretion
M	mol/l
MeOH	Methanol
MG	Molekulargewicht
min	Minute
mg	Milligramm
µl	Mikroliter
µM	Mikromolar
ORM	Orosmucoid
PBS	Phosphate buffered saline, Phosphatgepuffertes Salz
PPB	Plasmaprotein – Bindung
Ref	Referenz
rpm	revolutions per minute
sec	Sekunde
TQI	Transil^{XL} Quality Index
V_D	scheinbares Verteilungsvolumen

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12. Anhang

12.1. Ergänzende Tabellen

Die sich im Anhang befindlichen Tabellen weisen die Peakflächen der HPLC-Chromatogramme auf. Diese Areas wurden zur Berechnung in den Excel Arbeitsblättern der Transil^{XL} Software herangezogen.

Leere Felder entstanden durch einen kaum ersichtlichen Peak, bei dem auch nach Erhöhung der Sensitivität keine Fläche mehr berechnet und angezeigt werden konnte.

12.1.1. HSA raw data

test article	Well	Sample	Area/height
Doxorubicin	A-1	Ref 1	284,700
Doxorubicin	B-1	Well 1	276,900
Doxorubicin	C-1	Well 2	264,500
Doxorubicin	D-1	Well 3	240,000
Doxorubicin	E-1	Well 4	258,700
Doxorubicin	F-1	Well 5	242,900
Doxorubicin	G-1	Well 6	201,900
Doxorubicin	H-1	Ref 2	281,900
SL 5	A-2	Ref 1	1,500
SL 5	B-2	Well 1	1,100
SL 5	C-2	Well 2	
SL 5	D-2	Well 3	
SL 5	E-2	Well 4	
SL 5	F-2	Well 5	
SL 5	G-2	Well 6	
SL 5	H-2	Ref 2	
SL 4	A-3	Ref 1	2,400
SL 4	B-3	Well 1	1,300
SL 4	C-3	Well 2	0,900
SL 4	D-3	Well 3	0,500
SL 4	E-3	Well 4	0,400
SL 4	F-3	Well 5	
SL 4	G-3	Well 6	
SL 4	H-3	Ref 2	2,100
SL 6	A-4	Ref 1	69,600
SL 6	B-4	Well 1	60,100
SL 6	C-4	Well 2	60,600
SL 6	D-4	Well 3	43,200
SL 6	E-4	Well 4	42,400
SL 6	F-4	Well 5	24,000
SL 6	G-4	Well 6	16,500
SL 6	H-4	Ref 2	67,800
SL 7	A-5	Ref 1	4,700
SL 7	B-5	Well 1	3,800
SL 7	C-5	Well 2	3,300
SL 7	D-5	Well 3	2,000
SL 7	E-5	Well 4	1,900
SL 7	F-5	Well 5	1,300

SL 7	G-5	Well 6	0,900
SL 7	H-5	Ref 2	3,800
SL 14	A-6	Ref 1	90,700
SL 14	B-6	Well 1	95,500
SL 14	C-6	Well 2	71,700
SL 14	D-6	Well 3	
SL 14	E-6	Well 4	45,000
SL 14	F-6	Well 5	35,800
SL 14	G-6	Well 6	18,300
SL 14	H-6	Ref 2	83,300
SL 1	A-7	Ref 1	19,500
SL 1	B-7	Well 1	31,000
SL 1	C-7	Well 2	30,500
SL 1	D-7	Well 3	17,000
SL 1	E-7	Well 4	16,400
SL 1	F-7	Well 5	12,600
SL 1	G-7	Well 6	18,000
SL 1	H-7	Ref 2	8,800
SL 8	A-8	Ref 1	23,000
SL 8	B-8	Well 1	38,700
SL 8	C-8	Well 2	16,900
SL 8	D-8	Well 3	13,200
SL 8	E-8	Well 4	12,700
SL 8	F-8	Well 5	9,200
SL 8	G-8	Well 6	6,000
SL 8	H-8	Ref 2	34,900
SL 12	A-9	Ref 1	3,500
SL 12	B-9	Well 1	5,000
SL 12	C-9	Well 2	4,600
SL 12	D-9	Well 3	2,200
SL 12	E-9	Well 4	1,100
SL 12	F-9	Well 5	0,600
SL 12	G-9	Well 6	
SL 12	H-9	Ref 2	4,100
SL 9	A-10	Ref 1	99,000
SL 9	B-10	Well 1	85,000
SL 9	C-10	Well 2	84,100
SL 9	D-10	Well 3	64,100
SL 9	E-10	Well 4	65,600
SL 9	F-10	Well 5	46,500
SL 9	G-10	Well 6	33,000
SL 9	H-10	Ref 2	94,600
SL 2	A-11	Ref 1	13,200
SL 2	B-11	Well 1	10,600
SL 2	C-11	Well 2	10,500
SL 2	D-11	Well 3	8,000
SL 2	E-11	Well 4	7,900
SL 2	F-11	Well 5	4,200
SL 2	G-11	Well 6	2,900
SL 2	H-11	Ref 2	11,800
SL 3	A-12	Ref 1	1,250
SL 3	B-12	Well 1	1,500
SL 3	C-12	Well 2	1,800
SL 3	D-12	Well 3	1,370
SL 3	E-12	Well 4	0,900

SL 3	F-12	Well 5	0,750
SL 3	G-12	Well 6	
SL 3	H-12	Ref 2	1,400
SL 13	A-1	Ref 1	87,700
SL 13	B-1	Well 1	72,700
SL 13	C-1	Well 2	43,800
SL 13	D-1	Well 3	24,500
SL 13	E-1	Well 4	13,900
SL 13	F-1	Well 5	2,700
SL 13	G-1	Well 6	1,125
SL 13	H-1	Ref 2	143,000
SL 11	A-2	Ref 1	143,200
SL 11	B-2	Well 1	143,200
SL 11	C-2	Well 2	131,800
SL 11	D-2	Well 3	1149,000
SL 11	E-2	Well 4	103,000
SL 11	F-2	Well 5	79,800
SL 11	G-2	Well 6	45,200
SL 11	H-2	Ref 2	140,500
SL 10	A-3	Ref 1	19,100
SL 10	B-3	Well 1	4,400
SL 10	C-3	Well 2	4,180
SL 10	D-3	Well 3	4,500
SL 10	E-3	Well 4	3,300
SL 10	F-3	Well 5	2,000
SL 10	G-3	Well 6	1,500
SL 10	H-3	Ref 2	5,500

12.1.2. AGP raw data

test article	Well	Sample	Area/height
Doxorubicin	A-1	Ref 1	313,600
Doxorubicin	B-1	Well 1	253,000
Doxorubicin	C-1	Well 2	270,100
Doxorubicin	D-1	Well 3	256,000
Doxorubicin	E-1	Well 4	237,500
Doxorubicin	F-1	Well 5	250,000
Doxorubicin	G-1	Well 6	241,500
Doxorubicin	H-1	Ref 2	
SL5	A-2	Ref 1	8,300
SL5	B-2	Well 1	3,000
SL5	C-2	Well 2	3,500
SL5	D-2	Well 3	4,300
SL5	E-2	Well 4	4,200
SL5	F-2	Well 5	2,300
SL5	G-2	Well 6	3,400
SL5	H-2	Ref 2	5,600
SL4	A-3	Ref 1	2,400
SL4	B-3	Well 1	2,600
SL4	C-3	Well 2	3,800
SL4	D-3	Well 3	2,400
SL4	E-3	Well 4	2,700
SL4	F-3	Well 5	3,300
SL4	G-3	Well 6	4,300
SL4	H-3	Ref 2	3,800
SL6	A-4	Ref 1	81,300
SL6	B-4	Well 1	70,000
SL6	C-4	Well 2	58,000
SL6	D-4	Well 3	59,900
SL6	E-4	Well 4	51,800
SL6	F-4	Well 5	55,500
SL6	G-4	Well 6	52,900
SL6	H-4	Ref 2	70,500
SL7	A-5	Ref 1	5,100
SL7	B-5	Well 1	1,900
SL7	C-5	Well 2	1,100
SL7	D-5	Well 3	0,800
SL7	E-5	Well 4	0,800
SL7	F-5	Well 5	0,780
SL7	G-5	Well 6	1,100
SL7	H-5	Ref 2	5,600
SL14	A-6	Ref 1	108,500
SL14	B-6	Well 1	149,300
SL14	C-6	Well 2	143,600
SL14	D-6	Well 3	53,600
SL14	E-6	Well 4	45,900
SL14	F-6	Well 5	61,600
SL14	G-6	Well 6	16,600
SL14	H-6	Ref 2	135,200
SL1	A-7	Ref 1	27,100
SL1	B-7	Well 1	32,000
SL1	C-7	Well 2	25,600
SL1	D-7	Well 3	24,600

SL1	E-7	Well 4	27,000
SL1	F-7	Well 5	24,000
SL1	G-7	Well 6	23,600
SL1	H-7	Ref 2	31,400
SL8	A-8	Ref 1	28,800
SL8	B-8	Well 1	16,800
SL8	C-8	Well 2	13,800
SL8	D-8	Well 3	8,300
SL8	E-8	Well 4	7,800
SL8	F-8	Well 5	5,500
SL8	G-8	Well 6	3,700
SL8	H-8	Ref 2	21,600
SL12	A-9	Ref 1	18,600
SL12	B-9	Well 1	14,700
SL12	C-9	Well 2	13,900
SL12	D-9	Well 3	13,000
SL12	E-9	Well 4	10,300
SL12	F-9	Well 5	9,800
SL12	G-9	Well 6	7,800
SL12	H-9	Ref 2	16,700
SL9	A-10	Ref 1	174,400
SL9	B-10	Well 1	52,500
SL9	C-10	Well 2	58,500
SL9	D-10	Well 3	42,300
SL9	E-10	Well 4	39,000
SL9	F-10	Well 5	24,400
SL9	G-10	Well 6	17,200
SL9	H-10	Ref 2	91,500
SL2	A-11	Ref 1	21,000
SL2	B-11	Well 1	23,000
SL2	C-11	Well 2	20,700
SL2	D-11	Well 3	21,800
SL2	E-11	Well 4	20,000
SL2	F-11	Well 5	20,400
SL2	G-11	Well 6	20,000
SL2	H-11	Ref 2	24,600
SL3	A-12	Ref 1	1,800
SL3	B-12	Well 1	2,500
SL3	C-12	Well 2	2,100
SL3	D-12	Well 3	1,400
SL3	E-12	Well 4	1,900
SL3	F-12	Well 5	1,200
SL3	G-12	Well 6	1,400
SL3	H-12	Ref 2	1,900
SL 13	A-1	Ref 1	40,700
SL 13	B-1	Well 1	26,700
SL 13	C-1	Well 2	26,200
SL 13	D-1	Well 3	23,700
SL 13	E-1	Well 4	20,200
SL 13	F-1	Well 5	18,200
SL 13	G-1	Well 6	18,200
SL 13	H-1	Ref 2	43,800
SL 11	A-2	Ref 1	131,600
SL 11	B-2	Well 1	115,000
SL 11	C-2	Well 2	112,400

SL 11	D-2	Well 3	126,200
SL 11	E-2	Well 4	127,800
SL 11	F-2	Well 5	116,100
SL 11	G-2	Well 6	110,200
SL 11	H-2	Ref 2	131,500
SL 10	A-3	Ref 1	9,000
SL 10	B-3	Well 1	8,700
SL 10	C-3	Well 2	7,400
SL 10	D-3	Well 3	6,200
SL 10	E-3	Well 4	5,700
SL 10	F-3	Well 5	1,700
SL 10	G-3	Well 6	1,300
SL 10	H-3	Ref 2	5,300

12.1.3. PPB raw data

test article	Well	Sample	Area/height
Doxorubicin	A-1	Ref 1	267,700
Doxorubicin	B-1	Well 1	272,500
Doxorubicin	C-1	Well 2	239,100
Doxorubicin	D-1	Well 3	256,600
Doxorubicin	E-1	Well 4	224,000
Doxorubicin	F-1	Well 5	213,800
Doxorubicin	G-1	Well 6	184,900
Doxorubicin	H-1	Ref 2	270,500
SL 5	A-2	Ref 1	11,200
SL 5	B-2	Well 1	17,000
SL 5	C-2	Well 2	14,100
SL 5	D-2	Well 3	
SL 5	E-2	Well 4	
SL 5	F-2	Well 5	
SL 5	G-2	Well 6	
SL 5	H-2	Ref 2	9,000
SL 4	A-3	Ref 1	3,400
SL 4	B-3	Well 1	1,600
SL 4	C-3	Well 2	1,000
SL 4	D-3	Well 3	0,600
SL 4	E-3	Well 4	0,400
SL 4	F-3	Well 5	
SL 4	G-3	Well 6	
SL 4	H-3	Ref 2	3,600
SL 6	A-4	Ref 1	102,000
SL 6	B-4	Well 1	52,400
SL 6	C-4	Well 2	42,600
SL 6	D-4	Well 3	34,500
SL 6	E-4	Well 4	25,300
SL 6	F-4	Well 5	17,200
SL 6	G-4	Well 6	13,100
SL 6	H-4	Ref 2	76,200
SL 7	A-5	Ref 1	5,600
SL 7	B-5	Well 1	2,700
SL 7	C-5	Well 2	1,700
SL 7	D-5	Well 3	1,200
SL 7	E-5	Well 4	0,700
SL 7	F-5	Well 5	0,800
SL 7	G-5	Well 6	0,500
SL 7	H-5	Ref 2	6,500
SL 14	A-6	Ref 1	101,900
SL 14	B-6	Well 1	83,900
SL 14	C-6	Well 2	68,300
SL 14	D-6	Well 3	56,200
SL 14	E-6	Well 4	36,400
SL 14	F-6	Well 5	20,700
SL 14	G-6	Well 6	12,000
SL 14	H-6	Ref 2	109,700
SL 1	A-7	Ref 1	34,400
SL 1	B-7	Well 1	31,200
SL 1	C-7	Well 2	25,200
SL 1	D-7	Well 3	23,900

SL 1	E-7	Well 4	18,400
SL 1	F-7	Well 5	12,000
SL 1	G-7	Well 6	7,700
SL 1	H-7	Ref 2	23,100
SL 8	A-8	Ref 1	47,100
SL 8	B-8	Well 1	17,200
SL 8	C-8	Well 2	14,000
SL 8	D-8	Well 3	10,600
SL 8	E-8	Well 4	7,700
SL 8	F-8	Well 5	4,900
SL 8	G-8	Well 6	2,100
SL 8	H-8	Ref 2	44,300
SL 12	A-9	Ref 1	22,000
SL 12	B-9	Well 1	8,500
SL 12	C-9	Well 2	5,600
SL 12	D-9	Well 3	3,600
SL 12	E-9	Well 4	2,300
SL 12	F-9	Well 5	1,900
SL 12	G-9	Well 6	
SL 12	H-9	Ref 2	19,600
SL 9	A-10	Ref 1	99,200
SL 9	B-10	Well 1	80,800
SL 9	C-10	Well 2	68,500
SL 9	D-10	Well 3	53,600
SL 9	E-10	Well 4	36,900
SL 9	F-10	Well 5	22,400
SL 9	G-10	Well 6	18,700
SL 9	H-10	Ref 2	89,000
SL 2	A-11	Ref 1	31,900
SL 2	B-11	Well 1	20,700
SL 2	C-11	Well 2	17,200
SL 2	D-11	Well 3	13,700
SL 2	E-11	Well 4	9,200
SL 2	F-11	Well 5	4,500
SL 2	G-11	Well 6	4,200
SL 2	H-11	Ref 2	29,300
SL 3	A-12	Ref 1	2,600
SL 3	B-12	Well 1	1,600
SL 3	C-12	Well 2	1,570
SL 3	D-12	Well 3	1,200
SL 3	E-12	Well 4	0,800
SL 3	F-12	Well 5	
SL 3	G-12	Well 6	
SL 3	H-12	Ref 2	3,000
SL 13	A-1	Ref 1	46,800
SL 13	B-1	Well 1	21,400
SL 13	C-1	Well 2	14,000
SL 13	D-1	Well 3	10,900
SL 13	E-1	Well 4	5,700
SL 13	F-1	Well 5	
SL 13	G-1	Well 6	
SL 13	H-1	Ref 2	41,700
SL 11	A-2	Ref 1	145,000
SL 11	B-2	Well 1	119,700
SL 11	C-2	Well 2	111,400

SL 11	D-2	Well 3	100,500
SL 11	E-2	Well 4	89,400
SL 11	F-2	Well 5	69,500
SL 11	G-2	Well 6	50,100
SL 11	H-2	Ref 2	136,200
SL 10	A-3	Ref 1	11,100
SL 10	B-3	Well 1	5,800
SL 10	C-3	Well 2	3,700
SL 10	D-3	Well 3	2,600
SL 10	E-3	Well 4	2,100
SL 10	F-3	Well 5	1,200
SL 10	G-3	Well 6	2,500
SL 10	H-3	Ref 2	11,100

12.2. Curriculum Vitae

Persönliche Daten

Name:	Katharina Sagaischek
Eltern:	Mutter: Elisabeth Mayr, geb. 1965 (dipl. Krankenschwester)
	Vater: DI. Mag. Franz Sagaischek, geb. 1962 (Architekt)
Geburtsdatum:	11. Juni 1990
Geburtsort:	Wien
Staatsangehörigkeit:	ÖSTERREICH
Familienstand:	ledig

Ausbildung

1996-2000:	Volksschule in Pressbaum
2000-2003:	Gymnasium Sacré Coeur Pressbaum
2003-2008:	BG/BRG Purkersdorf
17.06.2008:	Reifeprüfung mit ausgezeichnetem Erfolg
10/2008 – 06/2014:	Studium an der Universität Wien: Diplomstudium Pharmazie

Studienbegleitende Tätigkeiten

2006-heute:	Nachhilfetätigkeiten in Deutsch, Englisch, Latein, Chemie
2008-2010:	Tutorin der Begabtenakademie NÖ Chemie BG/BRG Purkersdorf unter der Leitung von Mag. Erwin Klein
09/2008:	Ferialpraktikum: Stadtgemeinde Purkersdorf zur Nationalratswahl
07/2010:	Ferialpraktikum: Rotunden Apotheke, 1020 Wien
11/2010 - 08/2011:	Teilzeitarbeit: Rotunden Apotheke, 1020 Wien
01/2012 - 09/2013:	Teilzeitarbeit im Architekturbüro des Vaters
10/2013 - 06/2014:	Tutorin für das Labor Klinische Pharmazie & Diagnostik

Persönliche Fähigkeiten

Muttersprache:	Deutsch
Sonstige Sprachen:	Englisch in Wort und Schrift, Latein
Führerschein:	B

Soziale Fähigkeiten und Kompetenzen

Klassensprecherin (2006-2008) & Schulsprecherin (2007-2008)
Organisationsleiterin diverser Balleröffnungen und öffentlicher Veranstaltungen
Jugendmitarbeiterin der Pfarre Reindorf (2011-2013)