IGFBP-3 - ELISA

Enzyme Immunoassay for Quantitative Determination of

Human Insulin-like Growth Factor Binding Protein 3 (IGFBP-3)

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SYMBOLS

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Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

DIN EN ISO 15223-1

	Expiry date/ Verfallsdatum/ Date de péremption/ Data di scadenza/ Fecha de caducidad/ Data de validade/ Uiterste gebruiksdatum/ Udløbsdato/ Bäst före-datum/ Termin ważności/ Lejárati idő/ Čas exspirácie/ Doba exspirace/ Срок на годност/ Aegumiskuupäev/ Ημερομηνία λήξης/ Data de expirare/ Rok uporabe/ Viimeinen käyttöpäivä
	Consider instructions for use/ Bitte Gebrauchsanweisung beachten/ Consultez la notice d'utilisation/ Consultare le istruzioni per l'uso/ Consulte las instrucciones de uso/ Respeitar as instruções de utilização/ A.u.b de gebruiksaanwijzing volgen/ Se brugsanvisningen/ Läs anvisningarna före användning/ Proszę przeczytać instrukcję obsługi/ Vegye figyelembe a használati utasításban foglaltakat/ Postupujte podľa pokynov na použitie/ Dodržujte návod k použití/ Моля, спазвайте инструкцията за употреба/ Palun järgige kasutusjuhendit/ Λάβετε υπόψη σας τις οδηγίες χρήσης/ Vă rugăm să respectaţi instrucţiunile de utilizare/ Upoštevajte navodila za uporabo!/ Lue käyttöohje huolellisesti!
	Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnumme/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ Erä
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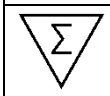
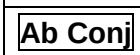
	Catalogue Number/ Bestellnummer/ Numéro de référence/ Numero di riferimento/ Número de referencia/ Número de Referência/ Referentienummer/ Referencenummer/ Beställningsnummer/ Numer katalogowy/ Rendelési szám/ Katalógové číslo/ Objednací číslo/ Каталожен номер/ Tellimisnumber/ Αρ. παραγγελίας/Număr comandă/ Številka naročila/ Viite tai tilausnumero
	Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. entre/ Armazenar entre/ Bewaar bij tussen/ Opbevares mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezí/ Температурно ограничение/ Säilitada temperatuuridel/ Φύλαξη σε θερμοκρασία/ Depozitare între/ Skladiščenje med/ Säilytys x-y Celsiusasteen lämpötilassa
	Contains sufficient for x tests/ Inhalt ausreichend für x Tests/ Contient suffisant pour x tests/ Contenuto sufficiente per x test/ Contenido suficiente para x pruebas/ Conteúdo suficiente para x testes/ Bevat voldoende voor x bepalingen/ Indeholder tilstrækkeligt til x prøver/ Innehållet räcker till x analyser/ Zawartość na x testów/ Tartalma x teszt elvégzésére elegendő/ Obsahuje materiál pre x testov/ Obsah dostačuje pro x testů/ Съдържание достатъчно за x тестове/ Sisust jätkub x katse jaoks/ Το περιεχόμενο επαρκεί για x δοκιμές/ Conţinut suficient pentru x teste/ Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille
	Incubation time/ Inkubationszeit/ Temps d'incubation/ Tempo d'incubazione/ Tiempo de incubación/ Tempo de incubação/ incubatietijd/ Inkubationstid/ inkubationstid/ Czas inkubacji/ Inkubációs idő/ Inkubačná lehota/ Inkubační doba/ Инкубационен период/ Inkubatsiooniaeg/ Χρόνος επώασης/ Timp de incubare/ Inkubacijska doba/ inkubaatioaika
	Incubate at/ Inkubation bei/ Incuber à/ Incubare a/ incubar a/ Incubar a/ incubatietemperatuur/ Inkubation ved/ inkubation vid/ Inkubacja przy/ Inkubáció hőmérséklete/ Inkubácia pri/ Inkubace při/ Инкубира се при/ Inkubatsioon temperatuuril/ Επώαση στους/ Incubare la/ Inkubacija pri/ Inkubaatiolämpötila
	Mix tubes with a Vortex mixer/ Mix Röhrchen mit Vortex Mixer/ Mélanger à l'aide d'un vortex/ Miscelare la provetta con agitatore Vortex/ Tubos de mezcla con mezclador de vórtex/ Misturar os tubos com um agitador Vortex/ buisjes mengen met een Vortex/ Blanderør med Vortex-mixer/ Blanda rören med en vortexblandare/ Miksowanie rurek w mikserze Vortex/ Csövecskék keverése örvénykeverővel/ Premiešať pomocou prístroja Vortex/ Promíchat pomocí přístroje Vortex/ Разбъркване на епруветките с миксер Vortex/ Segada torukesi Vortexi mikseriga/ Αναμίξτε τους σωληνίσκους με αναδευτήρα Vortex/ Amestecaţi eprubetele cu ajutorul unui agitator vortex/ Mešanje cevčic z mešalnikom Vortex/ Sekoita putket Vortex sekoittajalla
	Miktrotiterplate/ Mikrotiterplatte/ plaque de microtitrage/ Piastra di microtitolazione/ Placa de microtitulación/ Placa de Microtitulação/ Microtiterplaat/ Mikrotiterplade/ mikrotiterplatta/ microtiterplaat/ Płytka microtiter/ Mikrotiter lap/ Mikrotitračná podložka/ Mikrotitrační podložka/ Микротитърна плака/ Mikrotiiterplaat/ Τρυβλίο μικροτιτλοδότησης/ Microplacă/ Mikrotitrska plošča/ Mikrotitrauslevy
	Reconstitute in/ Rekonstituieren in/ Reconstituer dans/ Ricostituire nel/ Reconstituir en/ Reconstituir em/ Reconstitueren in/ Rekonstituér i/ Rekonstituera/ Rekonstytuować w/ Helyreállítás/ Znovu pripraviť za/ Znovu připravit za/ Разтваряне в/ Moodustada uuesti/ Ανασυστήστε σε/ Reconstituire în/ Predelava v/ Rekonstituoi
	Sample/ Probe/ Echantillon/ Campione/ Muestra/ Amostra/ Monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Проба/ Proov/ Δείγμα/ Probă/ Vzorec/ Näyte
	Antibody and Enzyme Conjugate/ Antikörper und Enzym Konjugat/ Anticorps conjuguée et conjugué enzymatique/ Coniugato di anticorpo ed enzima/ Conjugado de anticuerpos y enzimas/ Conjugado Anticorpo-Enzima/ Antilichaam- en enzymconjugaat/ Antistoffer og enzym-konjugat/ Antikropps- och enzymkonjugat (antikropp och enzym, konjugat)/ Koniugat antyciał i enzymów/ Antitest és enzim páros/ Protilátkový a enzymatický konjugát/ Protilátkový a enzymatický konjugát/ Антитяло и ензим конюгат/ Antikehad ja ensüümi konjugaat/ Σύμπλοκο αντισώματος-ενζύμου/ Compuşi din anticorpi şi enzime/ Antitelesa in konjugat encima/ Vasta-aine ja entsymi konjugaatti

BUF	Buffer/ Puffer/ Tampon/ Tampone/ Tampón/ Tampão/ Buffer/ Buffer/ Buffert/ Bufor/ Puffer/ Pufer/ Pufr/ Буфер/ Puhver/ Ρυθμιστικό διάλυμα/ Tampon/ Pufer/ Puskuri
DILU BUF X	Dilute in Buffer X/ Verdünnen in Puffer X/ Diluer dans le tampon X/ Diluire nel tampone X/ Diluir en tampón X/ Diluir no Tampão X/ Verdunnen in buffer X/ Fortyndes i buffer X/ Späd i buffert X/ Rozcieńczanie w buforze X/ Hígítás X pufferben/ Riediť v pufri X/Ředit v pufru X/ Разреждане в буфер X/ Lahjendada puhvris X/ Αραιώστε σε ρυθμιστικό
STD	Standard X/ Standard X/ Etalon X/ Standard X/ Estándar X/ Standard X/ Standaard X/ Standard X/ standard X/ Standard X/ Standard X/ Štandard X/ Standard X/ Стандарт X/ Standard X/ Πρότυπο X/ Standard X/ Standardni X/ Standardi X
Control	Control Serum X/ Kontrollserum X/ Contôle sérique X/ Siero di controllo X/ Suero de control X/ Soro de Controlo X/ controleserum X/ Kontrolserum X/ Kontrollserum X/ Serum kontrolne X/ Ellenőrző szérum X/ Kontrolné serum X/ Kontrolní serum X/ Контролен серум X/ Kontrollseerum X/ Ορός ελέγχου X/ Ser de control X/ Kontrolni serum X/ Kontrolli seerumi X
WASHBUF 20x	Washing Buffer Concentrate/ Waschpufferkonzentrat/ Tampon de lavage conc./ Tampone di lavaggio concentrato/ Tampón de lavado concentrado/ Tampão de Lavagem Concentrado/ wasbuffer, geconcentreerd/ Vaskebufferkoncentrat/ Vaskebufferkoncentrat/ tvättbuffertkoncentrat/ Bufor płukania koncentrat/ Mosópuffer koncentrátum/ Koncentrát vymývacieho pufra/ Концентрат на промивен буфер/ Pesupuhvri kontsentraat/ Συμπύκνωμα ρυθμιστικού διαλύματος έκπλυσης/ Concentrat pentru tamponul de spălare/ Koncentrat izpiralnega pufra/ Pesuliuositiiviste
WASHBUF	Washing Buffer/ Waschpuffer/ Tampon de lavage/ Tampone di lavaggio/ Tampón de lavado/Tampão de Lavagem/ wasbuffer/ Vaskebuffer/ tvättbuffert/ Bufor płukania/ Mosópuffer/ Vymývací pufer/ Vymývací pufr/ Промивен буфер/ Pesupuhver/ Ρυθμιστικό διάλυμα έκπλυσης/ Tampon pentru spălare /Izpiralni pufer/ Pesuliuos
SUBST TMB	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
H_2SO_4	Stopping Solution/ Stopplösung/ Stop Solution/ Soluzione di stop/ Stop Solución/ Solução Stop/ stopoplossing/ Stopopløsning/ Stopplösning/ Stop roztwór/ Megállító oldat/ Roztok na ukončenie/ Roztok pro ukončení/ Стопиращ разтвор/ Stopp-lahus/ Διάλυμα διακοπής/ Soluţie de oprire/ Stop raztopina/ Pysäytysliuos
TAPE	Cover Plate with sealing tape/ Platte abkleben/ Recouvrir la microplaque avec bande adhésive/ Coprire la piastra con nastro adesivo/ Cubrir la placa con una cinta adhesiva/ Cobrir a Placa com fita adesiva/ plaatje met tape afdekken/ Afdækningsplade med tape/ maskera platta/ Odkleić płytkę/ Tányér leragasztása/ Oblepiť podložku lepiacou páskou/ Olepit podložku lepicí páskou/ Плака с лента за запечатване/ Katta plaat isoleerkleeplindiga/ Κολλήστε το πλακίδιο με κολλητική ταινία/ Aoperiţi placa cu o bandă adezivă/ Prelepiti ploščo/ Peitä mikrotitrauslevy oheisella teipillä
MEASURE	Measure plate within 30 min at 450 nm (Referencefilter ≥590 nm)/ Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)/ Mesure lábsorbance en léspace de 30 min à 450 nm avec ≥590 nm longueur dónde pour référence/ Misurazione entro 30 min. a 450 nm (filtro di riferimento ≥ 590 nm)/ Medición de la placa dentro de los siguientes 30 min a 450 nm (filtro de referencia ≥ 590nm)/ Medir a placa dentro de 30 min a 450 nm (Filtro de referência ≥ 590 nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved nm (referencefilter ≥590 nm)/ Mät inom 30 min vid 450 nm (referensfilter ≥ 590 nm)./ Pomiar w ciągu 30 min przy 450 nm (filtr odniesienia ≥ 590 nm)/ Ki mérés 30 percen belül 450 nm-nél (referenciaszűrő ≥ 590 nm)/ Merať 30 minút pri 450 nm (Referenčných filtrov ≥590 nm)/ Měřit 30 minut při 450 nm (Referenční filtr ≥ 590 nm)/ Отчитане в рамките на 30 min при 450 nm (референтен филтър ≥ 590 nm)/ Mõõtmine 30 min jooksul 450 nm korral (võrdlusfilter ≥ 590 nm)/ Μέτρηση εντός 30 min στα 450 nm (φίλτρο αναφοράς ≥ 590 nm)/ Măsurare în decurs de 30 min la 450 nm (filtru de referinţă ≥ 590 nm)/ Izmerite ploščico v 30 min pri 450 nm (referenčni filter ≥590nm)/ Mittaa 30 minuutin aikana 450 nm:ssä (referenssi suodatin ≥ 590 nm)
Literatur	Literature/ Literatur/ Bibliographie/ Letterario/ Bibliografía/ Literatura documentação/ literatuur/ Litteratur/ litteratur/ Literatura/ Irodalom/ Literatúra/ Literatura/ Литература// Kirjandus/ Βιβλιογραφία/ Bibliografie/ literatura/ Lähdeluettelo
International Test description	International test description/ internationale Testanleitung/ description internationale de test/ Istruzioni per il test internazionali/ Descripción de ensayo internacional/ Descrição internacional do teste/ internationale testbeschrijving/ internationell testbeskrivning/ Opis testu międzynarodowego/ nemzetközi teszt-útmutató/ Medzinárodný návod k testu/ Mezinárodní návod k testu/ rahvusvaheline katse kirjeldus/ Διεθνείς οδηγίες για εργαστηριακές δοκιμές/ instrucţiuni internaţionale pentru testare/ mednarodna navodila za preizkus/ Kansainvälinen käyttöohje
End	in all required wells/ in allen benötigten Vertiefungen/ dans tous les godets requis/ in tutti i pozzetti richiesti/ en todos los pozos requeridos/ em todos os tubos necessários/ in alle nodige putjes/ i alle nødvendige brønde/ i alla nödvändiga brunnar/ we wszystkich potrzebnych wgłębieniach/ minden szükséges forrásban/ vo všetkých potrebných miestach/ ve všech potřebných místech/ във всички необходими ямки/ kõigis vajalikes süvendites/ σε όλες τις απαραίτητες κοιλότητες/ în toate cavităţile necesare/ v vseh zahtevanih vdolbinah/ kaikkiin tarvittaviin mikrotitrauslevyn syvennyyksiin

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For in vitro use only.
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Read entire protocol before use !

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ENGLISH Instructions for use

IGFBP-3 ELISA	96 Determinations
Principle of the test	Sandwich ELISA
Duration (incubation period)	2.5 h
Antibody-HRP-Conjugate	ready for use
Buffer and Substrate	ready for use
Standards	5 single standards: 0.4 - 30 ng/mL, lyophilized, human IGFBP-3
Assay Range	0.03 – 15150 ng/mL
Control	2 control sera, lyophilised
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:505
Analytical sensitivity	0.03 ng/mL
average Intra- / Inter-Assay Variance	1.9% / 5.7%
Reference Values	Blum W.F et.al.1990 Insulin-Like Growth Factors and Their Binding Proteins. In: Ranke MB, Mullins P.E.(ed): Diagnostics of Endocrine Function in Children and Adolescents. Basel, Karger, 2011, pp.157-181

INTRODUCTION

Insulin-like growth factors (IGF)-I and -II are bound to specific binding proteins (IGFBPs) in the circulation. To date, at least six binding proteins can be distinguished on the basis of their amino acid sequence. They are designated as IGFBP-1, IGFBP-2 to IGPBP-7 (1-2). The predominating IGFBP in blood is IGFBP-3, which largely determines the total IGF-I and IGF-II concentration. In contrast to the other binding proteins, IGFBP-3 has the property to associate with an acid-labile non-binding subunit (ALS) after binding of either IGF-I or IGF-II (3-5). Most of the IGFBP-3 in plasma is present as the high molecular weight ternary complex, however, small amounts of free IGFBP-3 are also found (6,7).

The development of specific immunoassays for IGFBP-3, recognizing the complete high molecular weight complex, provided new in-sights into ternary complex regulation (6-9).

Several factors besides GH influence IGFBP-3 levels: age including sexual development, nutrition, hypothyroidism, diabetes mellitus, liver function and kidney function.

Measurement over 24 hours revealed constant circadian levels (12,13).

INTENDED USE

This enzyme immunoassay kit is for research use and quantifies IGFBP-3 in human Serum, Heparin or EDTA plasma.

ASSAY PRINCIPLE

The Mediagnost ELISA for IGFBP-3 E03A is a so-called Sandwich-Assay. It utilizes two specific antibodies of high affinity. First the IGFBP-3 in the sample binds to the immobilized antibody on the microtiter plate. In the following step, the complex of biotinylated anti-IGFBP-3-Antibody and Streptavidin-Peroxidase binds in turn to the immobilised IGFBP-3. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the IGFBP-3 content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

SAMPLES

Sample type

Serum and Plasma
Serum and Heparin/EDTA Plasma yield comparable values.

Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions have to be avoided.

Required sample volume: 10 µL

Sample stability

In firmly closable sample vials

- Storage at 20-25°C: 3 days
- Storage at -20° C: min. 2 years
- Freeze-thaw cycles max. 10

The storage of samples over a period of 2 years at -20°C, showed no influence on the reading. Freezing and thawing of samples should be minimized. 10 Freezing-Thawing showed no effect on samples.

Interference

Triglyceride, bilirubin and hemoglobin in the sample do not interfere to a concentration of 100 mg/mL, 100 µg/mL or 5 mg/mL, respectively. However, the use of haemolytic, lipemic or icteric samples should be validated by the user.

Sample dilution

- Dilution: **1:505** with Sample Buffer **PP**
- Pipette **1 ml Sample Buffer PP** (red colored) in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µL Serum**- or **Plasma** (dilution factor 101). Add **400 µL** Sample Buffer **PP** in another PE-/PP-tube and **100 µL** of the thoroughly mixed first dilution (dilution factor 5). After mixing use **50 µL** of this 1:505 diluted solution **within 1 hour per determination** in the assay.
- Sample stability after dilution of the sample: maximum 1 hour at 20-25°C.

MATERIALS

Materials provided

The reagents listed below are sufficient for 96 wells including the standard curve.

MTP	**Microtiter plate,** ready for use, coated with rabbit-anti-hIGFBP-3-antibody. Wells are separately breakable.	**(8x12) wells**
A-E	**Standards**, lyophilized, (human IGFBP-3), concentrations are given on vial labels and on the QC-certificate.	**5 x 1 mL**
KS1	**Control Serum 1,** lyophilised, (human serum), concentration is given on the QC-certificate.	**1 x 250 µL**
KS2	**Control Serum 2,** lyophilised, (human serum), concentration is given on the QC-certificate.	**1 x 250 µL**
AK	**Antibody-HRP-Conjugate,** ready for use, contains rabbit biotinylated anti-hIGFBP-3 antibody**.**	**1 x 12 mL**
PP	**Sample Buffer,** red colored, ready for use, **Please shake before use!**	**1 x 120 mL**
VP	**Dilution Buffer,** ready for use, **Please shake before use!**	**1x 30 mL**
WP	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised H_2O_2 Tetramethylbencidine.	**1 x 12 mL**
SL	**Stopping Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
-	**Sealing Tape,** for covering the **microtiter plate.**	**2 x**
	**Instructions for use**	**1 x**
--	**Quality Control Certificate**	**1 x**

Materials required, but not provided

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer **WP (A. dest.),** 950 mL.
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

TECHNICAL NOTES

Storage Conditions

Store the kit at 2-8°C after receipt until its expiry date. The lyophilized reagents should be stored at –20 °C after reconstitution. Avoid repeated thawing and freezing.

Storage Life

The shelf life of the components **after initial opening** is warranted for **4 weeks**, store the unused strips and microtiter wells **airtight** together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The **reconstituted components** standards **A-E** and Control Sera **KS1** and **KS2** must be stored at –20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C

Preparation of reagents

Bring all reagents **to room temperature (20 - 25°C) before use**. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.

Reconstitution

The Standards **A – E** and Controls **KS1 and KS2** are reconstituted with the Sample Buffer **PP**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

Dilution

After reconstitution dilute the Control Sera **KS1 and KS2** with the Sample Buffer **PP** in the same ratio (1:505) as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

Incubation

Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution **S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive–store and incubation in the dark.

Shaking

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must be adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/ or false values, excessive shaking may result in high optical densities and/ or false values.

Washing

Proper washing is of basic **importance** for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided Washing Buffer **WP** diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

The danger of handling with potentially infectious material must be taken into account.

When using an **automatic microtiter** plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

WARNINGS AND PRECAUTIONS

For research and professional use only.

The Mediagnost kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Mediagnost will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

Human Serum

Following components contain human serum: **Control Sera KS, KS2 Standards A-E**

Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious.

Reagents AK, VP, WP

Contain as preservatives f **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

Substrate Solution (S)

The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stopping Solution (SL)

The Stopping solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

ASSAY PROCEDURE

NOTES: All determinations (Standards **A-E**, Control Sera **KS1/KS2** and **samples**) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
When performing the assay, the **Blank**, Standards **A-E**, Control Sera **KS1/KS2** and the **samples** should be pipetted as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, the Antibody-POD-Conjugate **AK**, the Substrate Solution **S** as well as the Stop Solution **SL** should be added to the plate in the same order and in the same time interval each, respectively.

1) Please pipette on before in **all needed wells 50 µL** Dilution Buffer **VP**.

2) Add **50 µL** Sample Buffer **PP** in positions A1/A2.

3) Pipette in positions B1/B2 **50 µL** each Standard **A (0.4 ng/mL)**,

   pipette in positions C1/C2 **50 µL** each Standard **B (2 ng/mL)**,

   pipette in positions D1/D2 **50 µL** each Standard **C (6 ng/mL),**

   pipette in positions E1/E2 **50 µL** each Standard **D (15 ng/mL),**

   pipette in positions F1/F2 **50 µL** each Standard **E (30 ng/mL).**

   To control the correct accomplishment **50 µL** of the 1:505 (or in respective dilution rate of the sample) in Sample Buffer **PP** diluted Control Sera **KS1** and **KS2** can be pipetted in positions G1/2 and H1/H2.

   Pipette **50 µL each** of the **diluted sample** (generally 1:505 diluted in Sample Buffer **PP**) in the rest of the wells, according to requirements. Please mix the dilutions immediately after sample addition and use within 60 minutes.

4) Cover the wells with the sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at 350 rpm).

5) After incubation aspirate the contents of the wells and wash the wells 5 times with **300 µL** Washing Buffer **WP**.

6) Following the last washing step pipette **100 µL** of the Antibody-POD-Conjugate **AK** in each well

7) Cover the wells with the sealing tape and incubate **1 hour** at **room temperature** (shake at 350 rpm).

8) After incubation wash the wells **5 times** with Washing Buffer **WP** as described in step 5).

9) Pipette **100 µL** of the TMB-Substrate solution **S** in each well.

10) Incubate the plate for **30 Minutes in the dark at** room **temperature**.

11) After incubation pipette **100 µL** Stop Solution **SL** in each well.

12) Measure the absorbance **within 30 minutes at 450 nm**

    (Reference filter ≥590 nm).

CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of standard E should be above 1.00.
Samples, which yield higher absorbance values than Standard E, should be re-tested with a higher dilution.

Establishing of the standard curve

The standards provided contain the following concentrations of hIGFBP-3

Standard	A	B	C	D	E
ng/mL	0.4	2	6	15	30

1) Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).
2) Subtract the mean absorbance of the blank from the mean absorbances of all other samples and standards.
3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4) Recommendation: Calculation of the standard curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **A higher-grade polynomial, or four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
5) The IGFBP-3 concentration in ng/mL (or pg/mL, according the chosen unit for the standards) of the samples can be calculated by **multiplication** with the respective **dilution factor.**

Example of a typical standard curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

	Blank	A	B	C	D	E
ng/mL	0.0	0.4	2	6	15	30
$OD_{(450\text{-}620\ nm)}$	0.204	0.254	0.453	0.911	1.706	2.390

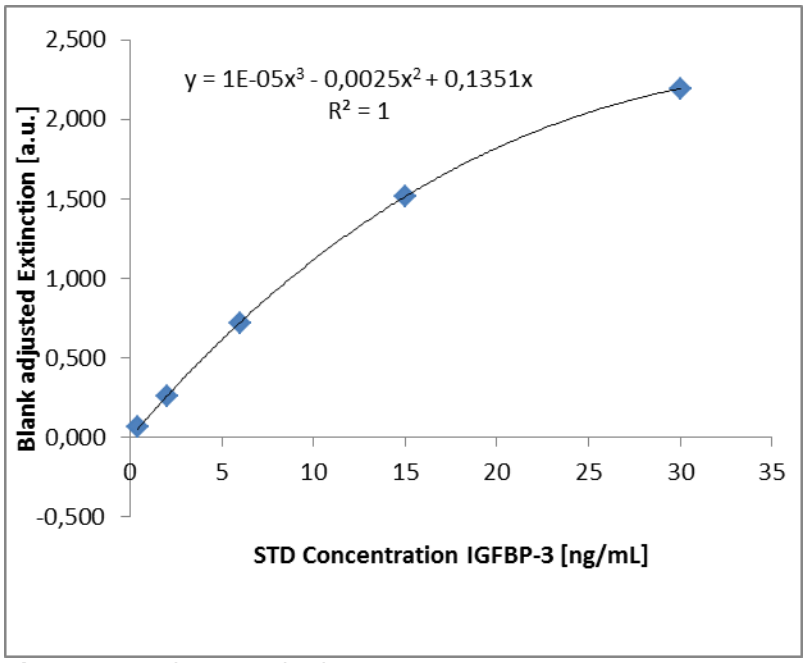

Fig. 1: Exemplary standard curve

The exemplary shown standard curve in Figure 1 **cannot** be used for calculation of your test results. You have to establish a standard curve for each test you conduct!

Exemplary calculation of IGFBP-3 concentrations

Sample dilution: 1:505

Measured extinction of your sample	0.975
Measured extinction of the blank	0.204

Your measurement program will calculate the IGFBP-3 concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial x^{rd} degree).

In this exemplary case the following equation is solved by the program to calculate the IGFBP-3 concentration in the sample:

$0.771 = 1E\text{-}05x^3 – 0.0025x^2 + 0.1351x$
$6.617 = x$

If the dilution factor (**1:505**) is taken into account the IGFBP-3 concentration of the undiluted sample is
6.617 ng/mL × 505 = 3342 ng/mL = 3,342 mg/L

PERFORMANCE CHARACTERISTICS

Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the 2fold standard deviation of the blank. The analytical sensitivity of the E03A is 0.03 ng/mL. According ICH Q2 R1 (CPMP/ICH/381/95) the limit of quantification (LoQ) is reflected by the recalculated IGFBP-3 concentration of the 10fold standard deviation of the blank, which therewith is 0.15 ng/mL.

Specificity

To determine the cross-reactivity of homologous proteins, the following proteins: IGFBP-1/4/5/6 were diluted to a concentration of 200 ng/mL in assay buffer and used as a sample in the assay. The relative cross-reactivities were on average: 0.11 / 0.14 / 0.17 / 0.1%.

Reproducibility and Precision

Intra-Assay-Variation

One sample has been measured 10 times in the same assay. The results are shown in table 1. The measured coefficient of variation (CV) is on average 1.9%

Tab. 1: Intra-Assay-Variation. Three exemplary serum samples were diluted and measured 10 times within one assay.

	Sample 1	Sample 2	Sample 3
Mean [ng/mL]	3630	3789	3016
SD	70.83	83.75	46.71
%CV	1.95	2.21	1.55
n	10	10	10

Inter-Assay-Variation

Serum samples were measured in independent assays on different days. On average the coefficient of variation was 5.7%. Results are shown in detail in table 2.

Tab. 2: Inter-Assay-Variation. Serum samples were diluted as recommended (1:505) and IGFBF-3 concentration was measured in different independent assays.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean [ng/mL]	2886	3525	3229	3219	4025	3293	3889	4328
SD	193	178	140	237	171	177	199	322
%CV	6.68	5.05	4.34	7.36	4.25	5.38	5.12	7.44
n	4	10	9	7	10	10	7	10

Linearity

Linearity was proven by dilution of three different serum samples with known IGFBP-3 concentration. The IGFBP-3 concentration of the diluted sample was measured and compared with the concentration expected. Results of linear regression analysis are shown in Figure 2. None of IGFBP-3 concentrations of the dilutions (1:125 to 1:2000) deviated more than 20% of the expected value (max. -17%).

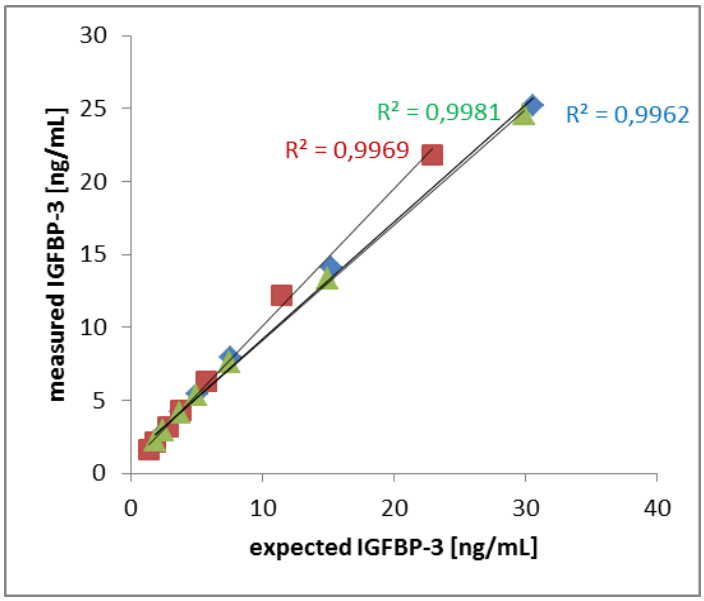

Fig. 2: Linearity. Shown are the measured concentrations in different dilutions of three serum samples.

Recovery

Serum and plasma samples were enriched with recombinant IGFBP-3 and the recovery was calculated in comparison to buffer enriched with the same amount of IGFBP-3. The native samples used had an IGFBP-3 concentration of 2684 to 3667ng/mL and the relative recovery was 109 – 118%. Results are shown in table 3.

Tab. 3: Recovery [%] of recombinant IGFBP-3 in native serum/plasma samples in comparison to recombinant IGFBP-3 in buffer.

IGFBP-3		Sample [ng/mL]	Sample enriched [ng/mL]	Target value [ng/mL]	Recovery [%]
Sample 1	Plasma	3641	5107	4324	118
Sample 2	Plasma	3667	4778	4350	110
Sample 3	Serum	2869	3778	3552	106
Sample 4	Serum	2684	3677	3367	109

Interference

Interference of physiological appearing substance with the IGFBP-3 measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering substances and the amount of IGFBP-3 was measured and compared with the IGFBP-3 concentration in the same sample without any enrichment. In table 4 the relative results are shown. None of the tested substances interfered significantly with IGFBP-3 measurement.

Tab. 4: Recovery [%] in comparison to the native serum.

	Triglyceride 100 mg/mL	Bilirubin 100 µg/mL	Hemoglobin 5 mg/mL
Sample 1	89	93	81
Sample 2	87	91	106
Sample 3	88	96	93

EXAMPLARY RESULTS

IGFBP-3-levels are strongly age-dependent in children, less so in adults. Exemplary results of IGFBP-3 concentrations in various age-groups which is log-normally distributed, are given in table 5. A graphic presentation is shown in Fig.3 and 4. It is recommended for each laboratory to establish its own normal range.

Tab. 5: Serum levels of IGFBP-3 in healthy subjects at various ages. Individuals between 7 and 17 years of age were classified according to gender.

		Percentiles													
Age group		**0.1**	1	**5**	10	**20**	30	**40**	50	**60**	70	**80**	90	**95**	99
0-1 week		**0.25**	0.33	**0.42**	0.48	**0.57**	0.64	**0.70**	0.77	**0.85**	0.93	**1.05**	1.23	**1.41**	1.81
1-4 weeks		**0.49**	0.62	**0.77**	0.86	**0.99**	1.10	**1.19**	1.29	**1.40**	1.52	**1.68**	1.93	**2.16**	2.68
1-3 months		**0.55**	0.70	**0.87**	0.98	**1.13**	1.25	**1.36**	1.48	**1.61**	1.75	**1.94**	2.23	**2.52**	3.14
3-6 months		**0.64**	0.80	**0.98**	1.10	**1.25**	1.38	**1.49**	1.61	**1.74**	1.88	**2.07**	2.37	**2.65**	3.24
6-12 months		**0.71**	0.88	**1.07**	1.19	**1.35**	1.48	**1.60**	1.72	**1.85**	2.00	**2.19**	2.49	**2.76**	3.36
1-3 years		**1.02**	1.21	**1.41**	1.53	**1.69**	1.82	**1.94**	2.05	**2.17**	2.31	**2.48**	2.74	**2.98**	3.47
3-5 years		**1.08**	1.30	**1.52**	1.66	**1.84**	1.99	**2.12**	2.25	**2.39**	2.55	**2.75**	3.05	**3.33**	3.91
5-7 years		**1.19**	1.42	**1.66**	1.81	**2.01**	2.16	**2.30**	2.44	**2.59**	2.76	**2.97**	3.29	**3.59**	4.2
7-9 y.	boys	**1.25**	1.48	**1.73**	1.88	**2.07**	2.22	**2.36**	2.50	**2.65**	2.81	**3.02**	3.33	**3.61**	4.22
	girls	**1.36**	1.61	**1.88**	2.04	**2.25**	2.42	**2.57**	2.72	**2.88**	3.06	**3.28**	3.62	**3.94**	4.58
9-11 y.	boys	**1.47**	1.73	**1.99**	2.15	**2.36**	2.52	**2.66**	2.81	**2.96**	3.14	**3.35**	3.67	**3.97**	4.57
	girls	**1.56**	1.90	**2.20**	2.38	**2.62**	2.80	**2.96**	3.13	**3.30**	3.50	**3.75**	4.11	**4.45**	5.16
11-13 y.	boys	**1.58**	1.88	**2.19**	2.38	**2.63**	2.82	**3.00**	3.18	**3.37**	3.58	**3.84**	4.25	**4.62**	5.39
	girls	**1.62**	1.90	**2.24**	2.46	**2.74**	2.97	**3.17**	3.38	**3.60**	3.85	**4.17**	4.65	**5.10**	6.02
13-15 y.	boys	**1.62**	1.89	**2.24**	2.46	**2.76**	2.99	**3.20**	3.42	**3.65**	3.91	**4.24**	4.75	**5.22**	6.20
	girls	**1.69**	2.03	**2.39**	2.61	**2.91**	3.14	**3.35**	3.56	**3.79**	4.04	**4.36**	4.85	**5.30**	6.24
15-17 y.	boys	**1.70**	2.02	**2.36**	2.57	**2.84**	3.05	**3.25**	3.44	**3.65**	3.88	**4.17**	4.61	**5.01**	5.86
	girls	**1.62**	1.93	**2.26**	2.46	**2.73**	2.93	**3.12**	3.31	**3.51**	3.74	**4.02**	4.45	**4.85**	5.67
17-20 y.		**1.58**	1.90	**2.24**	2.45	**2.72**	2.94	**3.13**	3.33	**3.54**	3.78	**4.07**	4.53	**4.95**	5.83
20-30 y.		**1.55**	1.86	**2.20**	2.41	**2.68**	2.90	**3.09**	3.29	**3.50**	3.74	**4.04**	4.50	**4.92**	5.80
30-40 y.		**1.44**	1.75	**2.08**	2.29	**2.56**	2.78	**2.98**	3.18	**3.39**	3.64	**3.95**	4.42	**4.86**	5.78
40-50 y.		**1.38**	1.68	**2.01**	2.21	**2.48**	2.69	**2.88**	3.08	**3.29**	3.53	**3.83**	4.29	**4.72**	5.63
50-60 y.		**1.34**	1.64	**1.96**	2.16	**2.42**	2.63	**2.83**	3.02	**3.23**	3.46	**3.76**	4.22	**4.65**	5.55
60-70 y.		**1.28**	1.58	**1.90**	2.10	**2.37**	2.58	**2.78**	2.98	**3.19**	3.44	**3.75**	4.23	**4.67**	5.62
70-80 y		**1.20**	1.50	**1.81**	2.00	**2.27**	2.47	**2.67**	2.87	**3.08**	3.32	**3.62**	4.09	**4.52**	5.44
> 80 y		**1.13**	1.43	**1.73**	1.92	**2.19**	2.39	**2.59**	2.79	**3.00**	3.23	**3.54**	4.00	**4.44**	5.36

Serum levels are given as mg/L
y. = years

Determined with IGFBP-3 RIA (Blum et al. 1990)
The values above 70 years are extrapolated.

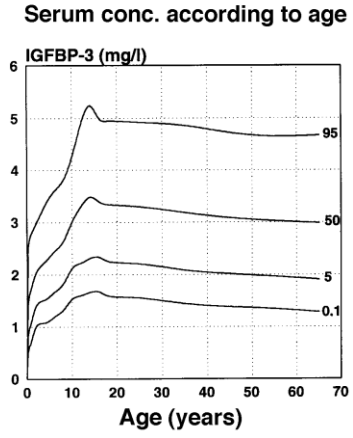

Fig.3: Age-dependant normal values of IGFBP-3 (presented as 0.1., 5., 50., and 95. percentile)

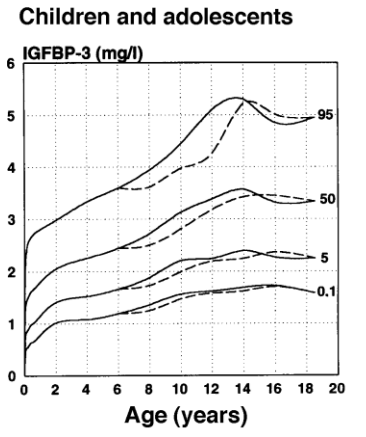

Fig.4: Normal values of children and adolescents (girls —— boys - - - -)

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SUMMARY OF THE ASSAY PROCEDURE E03A

Preparation of reagents		**Reconstitution:**	**Dilution**
A-E	**Standards**	in **1 mL** Sample Buffer **PP**	-
KS1	**Control Serum 1**	in **250 µL** Sample Buffer **PP**	**1:505** with **PP**
KS2	**Control Serum 2**	in **250 µL** Sample Buffer **PP**	**1:505** with **PP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**
Sample dilution: with Sample Buffer **PP 1:505**			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay Procedure in Double Determination:

Pipette	**Reagents**	**Position**
50 µL	Dilution Buffer **VP**	Pipette **in <u>all</u> required number of wells**
50 µL	Sample Buffer **PP** as Blank	A1/A2
50 µL	Standard **A** (**0.4 ng/mL**)	B1/B2
50 µL	Standard **B** (**2 ng/mL**)	C1/C2
50 µL	Standard **C** (**6 ng/mL**)	D1/D2
50 µL	Standard **D** (**15 ng/mL**)	E1/E2
50 µL	Standard **E** (**30 ng/mL**)	F1/F2
50 µL	Control Serum **KS 1** (1:505 diluted)	G1/G2
50 µL	Control Serum **KS 2** (1:505 diluted)	H1/G2
50 µL	Sample (1:505 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: 1 h at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WP/ well**	In each well
100 µL	Antibody-POD-Conjugate **AK**	In each well
Cover the wells with the sealing tape.		
Incubation: 1 hour at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WP/ well**	In each well
100 µL	Substrate Solution **S**	In each well
Incubation: 30 Minutes in the Dark at 20-25°C		
100 µL	Stopping Solution **SL**	In each well
	Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.	

Internationale Assay Description

A-E	**STD**	**Rec in 1 mL BUF PP**	**-**
KS1	**Control**	**Rec in 250 µL BUF PP**	**1:505 DILU BUF PP**
KS2	**Control**	**Rec in 250 µL BUF PP**	**1:505 DILU BUF PP**
WP	**WASHBUF 20x**	**-**	**1:20 DILU A. dest.**
-	**SPE**		**1:505 DILU BUF PP**
-	**°C 20-25 °C**		

50 µL	**BUF VP**	A1 - End
50 µL	**BUF PP**	A1/A2
50 µL	**STD A (0.4ng/mL)**	B1/B2
50 µL	**STD B (2 ng/mL)**	C1/C2
50 µL	**STD C (6 ng/mL)**	D1/D2
50 µL	**STD D (15 ng/mL)**	E1/E2
50 µL	**STD E (30 ng/mL)**	F1/F2
50 µL	**CONTROL KS1 1:505 DILU BUF PP**	G1/G2
50 µL	**CONTROL KS2 1:505 DILU BUF PP**	H1/H2
50 µL	**SPE 1:505 DILU BUF PP**	
	TAPE	
	1 h °C 20-25 ↔ 350 rpm	
5x 300 µL	**5x WASHBUF WP**	
100 µL	**AbCONJ AK**	
	TAPE	
	1 h °C 20-25 ↔ 350 rpm	
5x 300 µL	**5x WASHBUF WP**	
100 µL	**SUBST TMB S**	
	0.5 h °C 20-25	
	H_2SO_4 SL	
	MEASURE	