IGFBP-1 ELISA

Enzyme Immunoassay for Quantitative Determination of

human Insulin-like Growth Factor Binding Protein 1 (IGFBP-1)

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

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↙ 2-8°C X 96 wells ↓

hE01

: Aspenhaustr. 25 • D-72770 Reutlingen / Germany
Phone: + 49 - (0) 7121 51484-0 • Fax: + 49 - (0) 7121 51484-10
E-Mail: contact@mediagnost.de • http://www.mediagnost.de

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	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 Conjugate/ Antikörperkonjugat/ Anticorps conjuguée/ Coniugato di anticorpo/ Conjugado de anticuerpos/ Conjugado anticorpo/ Antilichaamconjugaat/ Antistoffer-konjugat/ Antikroppskonjugat/ Koniugat antyciał/ Antitest páros/ Protilátkový konjugát/ Protilátkový konjugát/ Антитяло конюгат/ Antikehad konjugaat/ Σύμπλοκο αντισώματος/ Compuşi

CONJ	Enzyme Conjugate/ Enzymkonjugat/ Conjugué enzymatique/ Coniugato di enzima/ Conjugado de enzimas/ Conjugado Enzima/ Enzymconjugaat/ Enzym-konjugat/ Enzymkonjugat/ Koniugat enzymów/ Enzim páros/ Enzymatický konjugát/ Enzymatický konjugát/ ензим конюгат/ Ensüümi konjugaat/ Σύμπλοκο –ενζύμου/ Compuşi din enzime/ Encima konjugat/ Enzymi-konjugaatti
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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ENGLISH Instructions for use

IGFBP-1 ELISA, E01	96 Determinations
Principle of the test	Enzyme-linked Immunoassay
Duration (incubation period)	1.75 h
Antibodies	specific, monoclonal antibodies
Buffer	Ready for use and 20fold concentrate
Standards	7 single standards: 0 - 8 ng/mL, lyophilized, native human IGFBP-1
Assay Range	0 – 128 ng/mL
Control	2 control sera, lyophilised
Sample	human serum / plasma
Required sample volume	20 µL
Sample dilution	≥ 1:16
Analytical sensitivity	< 0.1 ng/mL
Intra- / Inter-Assay Variance	Ø < 10%

1 INTENDED USE

This enzyme immunoassay kit is for **research use only** and measures IGFBP-1 in human serum or Heparin and EDTA plasma or in other body fluids, for example amnion fluid, mother milk, urine or saliva. It is also suited to quantify IGFBP-1 in cell culture media.

2 INTRODUCTION

The Insulin-like Growth Factors I and – II are free in body fluids and tissues but are bound to specific binding proteins. Until today seven different binding proteins (IGFBP-1 to –7) can be differentiated additionally several IGFBP-related proteins have also been detected. Bioavailability of IGF is regulated by these IGFBPs or better their proteolytic cleavage which reduces affinity to IGF. But the IGFBPs as well as their proteolytic fragments can also exert IGF-independent effects, like influencing cell migration or proliferation.

IGFBP-1 (Placental Protein 12) consists of 234 aminoacids and has a molecular weight of approximately 25kDa. The coding DNA region is located on chromosome 7. IGFBP-1 is mainly synthesized by foetal and adult liver tissue and decidual endometrium. Intensity of Expression varies enduring menstruation with a maximal expression in the late secretory phase. IGFBP-1 is posttranslational modified by phosphorylation of serine residues 101, 119 and 169. Phosphorylation has physiological relevance as it increases affinity of IGFBP-1 to IGF. In adult humans phosphorylated IGFBP-1 of the liver is the predominant form in circulation. IGFBP-1 produced by endometrial tissue is significantly less phosphorylated than the liver originated form

3 ASSAY PRINCIPLE

The Mediagnost ELISA for IGFBP-1 E01 is a so-called Sandwich-Assay. It utilizes two specific antibodies of high affinity. First the IGFBP-1 in the sample binds to the immobilized antibody on the microtiter plate. In the following step, the anti-IGFBP-1-Antibody binds in turn to the immobilised IGFBP-1. This is biotinylated and allows the binding of a streptavidin-peroxidase enzyme conjugate.

Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the IGFBP-1 content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

4 WARNINGS AND PRECAUTIONS

For in-vitro use only. For Professional use only.

The Mediagnost kit is suitable only for in vitro use and not for internal use in humans and animals, FOR RESEARCH USE ONLY. 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 KS1 / 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.

H- and P statements refer to undiluted substances and are for information purpose.

Reagents AK, EK, VP, WP
Contain as preservative **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.

4.1 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.

5 SAMPLES

5.1 Sample type

Beside serum also EDTA- and Heparin Plasma can be used, as well as the samples listed in the table 1.

5.2 Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions have to be avoided. For blood sampling diurnal variations specially influenced by nutrition should be considered [5].

5.3 Required sample volume: 20 µL

5.4 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. 3

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. 3 Freezing-Thawing showed no effect on samples.

5.5 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.

5.6 Sample dilution

- Dilution: ≥**1:16** with Dilution Buffer **VP**
- Pipette **300 µL** Dilution Buffer **VP** (red colored) in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **20 µL Serum**- or **Plasma** (dilution factor 16). After mixing use **50 µL** diluted solution **within 1 hour per determination** in the assay.

5.7 Other Body fluids

Where required, depending on the expected IGFBP-1-values, the dilution with Dilution Buffer VP can be higher or lower (at least however 1:2.5). The IGFBP-1 concentration may be completely variable in different body fluids. Examples as well as dilution recommendations are given in table 1.

Table 1 Results of matrix tests. Purified native IGFBP-1 was added to the respectively diluted samples. Enriched samples were measured without further dilution. Shown is the relative recovery of added IGFBP-1 of the value measured in enriched Dilution Buffer.

Samples	Expected IGFBP-1 [ng/ml]	Recovery IGFBP-1 [%]	Recommended Dilution
Amniotic Fluid	8.140 - 16.450	n.d.	≥1:5000 up to 1:25000
Mother´s milk	5.12	96	1:10
Urine	0.07	90	1:2.5
Saliva	< 0.02	63	≥1:2.5
Bronchial Lavage	< 0.02	100	1:2.5
Sputum	< 0.02	100	1:20

Cell Culture Media	---	94	≥1:5

n.d.= not determined

6 MATERIALS

6.1 Materials provided

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

MTP	**Microtiter plate,** ready for use, coated with mouse IGFBP-1-antibody. Wells are separately breakable.	**(8x12) wells**
A-G	**Standards**, lyophilized, (native human IGFBP-1), concentrations are given on vial labels and on the QC-certificate.	**7 x 500 µL**
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 Conjugate,** ready for use, contains mouse biotinylated anti-hIGFBP-1 antibody**.**	**1 x 6 mL**
EK	**Enzyme Conjugate EK,** contains HRP (Horseradish-Peroxidase)-labeled Streptavidin.	**1 x 12 mL**
VP	**Dilution Buffer,** ready for use, **Please shake before use!**	**1x 125 mL**
WP	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised 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**

6.2 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

7 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-G** and Control Sera **KS1** and **KS2** must be stored at –20°C. 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 stable for **4 weeks** at **2-8°C**

Preparation of reagents

Bring all reagents to room temperature (20°C - 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 – G** and Controls **KS1 and KS2** are reconstituted with the Dilution Buffer **VP**. 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 Dilution Buffer **VP** in the same ratio as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

Assay Procedure

When performing the assay, Standards **A-G**, Control Serum **KS1 and KS2** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate **AK,** Enzyme Conjugate **EK** as well as the succeeding Substrate Solution **S** should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution **SL** should be added to the plate in the same order as Substrate Solution **S**.
All determinations (Standards **A-G**, Control Sera **KS1 and KS2** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

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.

8 ASSAY PROCEDURE

Preparation of reagents		**Reconstitution:**	**Dilution**
A-G	**Standards**	in **500 µL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **250 µL** Dilution Buffer **VP**	**≥ 1:16** with **VP**
KS2	**Control Serum 2**	in **250 µL** Dilution Buffer **VP**	**≥ 1:16** with **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**
Sample dilution: with Dilution Buffer **VP ≥ 1:16**			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay Procedure in Double Determination:

Pipette	**Reagents**	**Position**
50 µL	Antibody Conjugate **AK**	Pipette **in <u>all</u> required number of wells**
50 µL	Standard **A** (**0 ng/mL**)	A1/A2
50 µL	Standard **B** (**0.1 ng/mL**)	B1/B2
50 µL	Standard **C** (**0.5 ng/mL**)	C1/C2
50 µL	Standard **D** (**1 ng/mL**)	D1/D2
50 µL	Standard **E** (**2 ng/mL**)	E1/E2
50 µL	Standard **F** (**4 ng/mL**)	F1/F2
50 µL	Standard **G** (**8 ng/mL**)	G1/G2
50 µL	Control Serum **KS 1** (**≥ 1:16** diluted)	H1/G2
50 µL	Control Serum **KS 2** (**≥ 1:16** diluted)	A3/A4
50 µL	Sample (**≥ 1:16** diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation <u>with Shaking</u>: 1 h at 20°C - 25°C, <u>350 rpm</u>		
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	Enzyme Conjugate **EK**	In each well
Cover the wells with the sealing tape.		
Incubation <u>with Shaking</u>: 30 Minutes at 20°C - 25°C, <u>350 rpm</u>		
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: 15 Minutes in the Dark at 20°C - 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.

9 CALCULATION OF RESULTS

9.1 Establishing of the standard curve

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

The standards provided contain the following concentrations of hIGFBP-1

Standard	**A**	**B**	**C**	**D**	**E**	**F**	**G**
ng/ml	0	0.1	0.5	1	2	4	8
OD (450-620 nm)	0.012	0.043	0.189	0.381	0.771	1.596	2.748

1) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.

2) 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).

3) The IGFBP-1 concentration in ng/mL of the samples can be calculated by **multiplication** with the respective **dilution factor.**

9.2 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. 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!

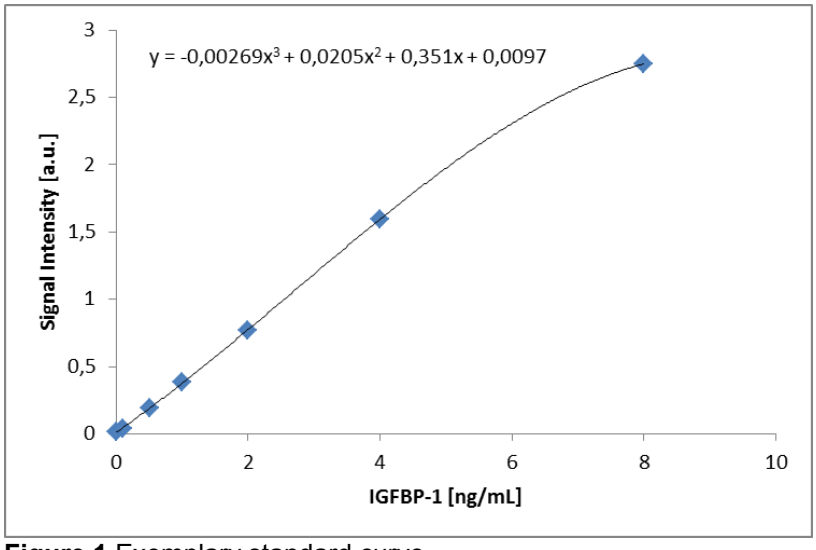

Figure 1 Exemplary standard curve

9.3 Exemplary calculation of IGFBP-1 concentrations

Sample dilution: 1:16
Measured extinction of your sample: 0.199

Your **measurement program** will calculate the IGFBP-1 concentration of the diluted sample automatically. You only have to determine the most suitable curve fit (here: polynomial 3rd degree). In this exemplary case the following equation is solved by the program to calculate the IGFBP-1 concentration in the sample:

$y = -0.00269x^3 + 0.0205x^2 + 0{,}351x + 0.0097$

$0.199 = -0.00269x^3 + 0.0205x^2 + 0.351x + 0.0097$

$x = 0.5235$

If the dilution factor (**1:16**) is taken into account the IGFBP-1 concentration of the undiluted sample is 0.5235 ng/mL × 16 = 8.376 ng/mL

9.4 Interpretation of results

Further, it is recommended to establish reference and cut-off values corresponding to the relevant group of pronband for each laboratory.

10 LIMITATION OF PROCEDURE

The Mediagnost IGFBP-1 ELISA, E01 is based on murine antibodies. Generally, this technique is sensible to heterophilic antibodies as well as to human anti mouse antibodies in the sample. The influence of these antibodies is reduced by assay design, but cannot be excluded completely. Further, for interpretation of IGFBP-1 concentrations sensitivity and specificity must be taken into account. Also test duration has to be considered, this test system is not suitable for point-of care.

11 EXEMPLARY VALUES

Concentrations of IGFBP-1 in human sera of 69 healthy adult donors were determined with the Mediagnost ELISA E01. Slight gender dependent differences were found, the concentrations of all samples varied from minimal 0.23 ng/ml to maximal 17.94 ng/ml (see table 1).

Table 1 IGFBP-1 Exemplary values in sera of healthy adults (measured values in **ng/ml**)

Gender	No. of Samples	Average value	Median	Min. – Max.:
female	33	4.79	4.24	0.23 – 16.07
male	36	5.22	2.71	0.42 – 17.94
total	69	5.01	2,77	0.23 – 17.94

12 PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

The analytical sensitivity was determined by measuring the null standard (STD-A) and calculating the corresponding concentration of the signal intensity of the STD-A + 2SD. In three different assays analytical sensitivities from 0.03 to 0.08 ng/mL were measured with an average value of 0.055 ng/mL

12.2 Specificity

The IGF/IGFBP-System consists of several related and homologous proteins. Thus the cross reactivity of the Mediagnost E01 IGFBP-1 ELISA to other IGFBPs was tested exemplarily with the two IGFBPs most abound in circulation. Table 2 summarizes the results.

Table 2 Cross reactivity to related IGFBPs. 500 ng of the indicated IGFBP were added to 1mL dilution buffer and measured as a sample. Shown is the concentration measured and the relative cross reactivity.

	Concentration [ng/ml]	**relative cross reactivity [%]**
IGFBP-2	0.0071	0.00142
IGFBP-3	0.0033	0.00066

12.3 Reproducibility and Precision

Intra-Assay-Variation

Serum samples were measured up to 20 times in the same assay and the coefficient was calculated based on the recalculated IGFBP-1 concentration. Exemplary data are shown in Table 3. The mean measured coefficient of variation (CV) is 6.52% (n =6).

Table 3 Intra-Assay Variation

	Sample 1	**Sample 2**	**Sample 3**
Mean [ng/mL]	4.25	12.21	55.17
SD [ng/mL]	0.24	0.75	2.38
CV [%]	5.59	6.11	4.32
Number [n]	20	20	20

Inter-Assay Variance

Serum samples were measured in independent assays. On average the coefficient of variation was 6.05% (SD 0.46). Exemplary results are shown in table 4.

Table 4: Inter-Assay Variation

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Mean [ng/mL]	12.50	8.52	6.42	18.62	4.43	9.01
SD [ng/mL]	0.17	0.24	0.25	0.52	0.18	0.18
CV [%]	1.33	2.80	3.90	2.80	4.11	2.05
Number [n]	5	5	5	5	5	5

12.4 Linearity

The linearity of sample dilution is shown in Figure 1. Exemplarily three serum sample were diluted 1:5 up to 1:512 and the IGFBP-1 concentration was measured within each dilution. The results were analysed by linear regression analysis and revealed coefficients of determination of >0.98 for each of the samples.

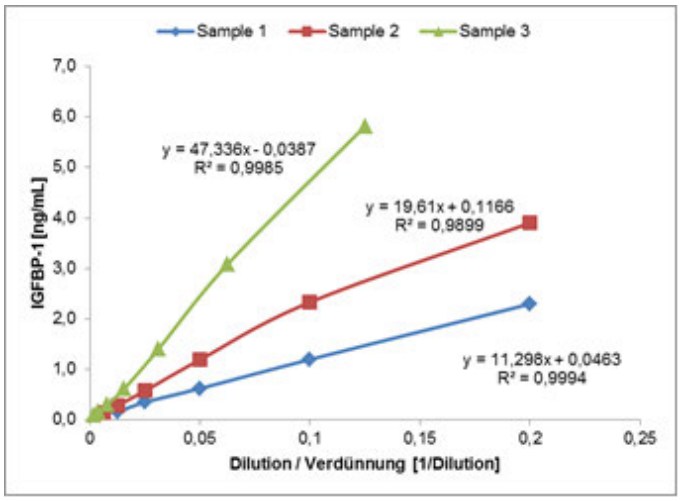

Figure 2 Linearity of the sample dilution

12.1 Recovery

Native IGFBP-1 (4.5 ng/mL) was added to human serum and quantified in comparison to the same amount of IGFBP-1 in buffer. The mean recovery detected was 82%. Also, the IGFBP-1 content of samples enriched with recombinant IGFBP-1 was measured and recovery calculated in comparison to enriched buffer (45 ng/mL) with a mean recovery of 94%.

12.2 Trueness / Assay calibration

There is no IGFBP-1 reference preparation available. Mediagnost IGFBP-1 ELISA E01 was calibrated against purified, native human IGFBP-1. Traceability / lot-to-lot consistency is accomplished by a panel of different serum samples.

12.3 Interference

Interference of haemoglobin, bilirubin and triglycerides was tested by adding the indicated amount of these substances to human serum containing IGFBP-1. For comparison the same amount of buffer without any substance was also added to the serum and the relative recovery rate was calculated. Table 5 shows that on average neither haemoglobin, bilirubin nor triglycerides exert significant influence on the measurement of IGFBP-1 in human serum. But generally measurement of IGFBP-1 in haemolytic, icteric or lipaemic samples should be avoided.

Table 5 Interference of haemoglobin. bilirubin and triglycerides on IGFBP-1 measurement in human serum. Shown is the relative recovery of IGFBP-1 measured in human serum samples after adding the indicated amount of possibly interfering substances in comparison to serum samples only buffer was added to.

Recovery [%]	Triglyceride 100 mg/mL	Bilirubin 100 µg/mL	Haemoglobin 5 mg/mL
Sample 1	142	72	122
Sample 2	125	46	119
Sample 3	82	113	97
Mean	**116**	**77**	**113**

13 ASSAY COMPARISON

Mediagnost IGFBP-1 E01 was compared with two competitor assays. The correlation of these test systems and the Mediagnost assay was evaluated by Passing-Bablok regression and linear regression. The results are shown in Table 6.

Tabelle 6 Assay comparison

	Sample [n]	Passing Bablok Regression	Linear Regression coefficient of regression
Competitor A	36	y = -0.99 + 0.3x (RSD 2.97)	0.89
Competitor B	34	y = 0.1 + 1.17x (RSD 0.84)	0.84

RSD= residual standard deviation

The analysis reveals that the Mediagnost test correlates well with both of the competitive tests. But regarding the absolute concentrations measured, there is a significant difference especially in comparison to competitor A. This indicates differences in calibration or binding properties of the antibodies used.

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15 INTERNATIONAL ASSAY PROCEDURE

A-G	**STD**	**Rec in 500 µL BUF VP**	**-**
KS1	**Control**	**Rec in 250 µL BUF VP**	**≥ 1:16 DILU BUF VP**
KS2	**Control**	**Rec in 250 µL BUF VP**	**≥ 1:16 DILU BUF VP**
WP	**WASHBUF 20x**	**-**	**1:20 DILU A. dest.**
-	**SPE**		**≥ 1:16 DILU BUF VP**
-	**°C 20°C -25 °C**		

50 µL	**Ab AK**	A1/A2 - End
50 µL	**STD A (0 ng/mL)**	A1/A2
50 µL	**STD B (0.1 ng/mL)**	B1/A2
50 µL	**STD C (0.5 ng/mL)**	C1/C2
50 µL	**STD D (1 ng/mL)**	D1/D2
50 µL	**STD E (2 ng/mL)**	E1/E2
50 µL	**STD F (4 ng/mL)**	F1/F2
50 µL	**STD G (8 ng/mL)**	G1/G2
50 µL	**CONTROL KS1 ≥ 1:16 DILU BUF VP** ↔	H1/H2
50 µL	**CONTROL KS2 ≥1:16 DILU BUF VP** ↔	A3/A4
50 µL	**SPE ≥1:16 DILU BUF VP** ↔	
TAPE		
1 h °C 20°C - 25°C ↔ 350 rpm		
5x 300 µL	**5x WASHBUF WP**	
100 µL	**CONJ EK**	A1/A2→ End
TAPE		
0.5 h °C 20°C - 25°C ↔ 350 rpm		
5x 300 µL	**5x WASHBUF WP**	
100 µL	**SUBST TMB S**	A1/A2→ End
0.25 h °C 20°C - 25°C		
100 µL	**H_2SO_4 SL**	A1/A2→ End
MEASURE		

16 ASSAY PROCEDURE

Preparation of reagents		Reconstitution:	Dilution
A-G	**Standards**	in **500 µL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **250 µL** Dilution Buffer **VP**	**≥ 1:16** with **VP**
KS2	**Control Serum 2**	in **250 µL** Dilution Buffer **VP**	**≥ 1:16** with **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**

Sample dilution: with Dilution Buffer **VP ≥ 1:16**

Before assay procedure bring all reagents to room temperature **20-25°C**.

Assay Procedure in Double Determination:

Pipette	Reagents	Position
50 µL	Antibody Conjugate **AK**	Pipette **in <u>all</u> required number of wells**
50 µL	Standard **A** (**0 ng/mL**)	A1/A2
50 µL	Standard **B** (**0.1 ng/mL**)	B1/B2
50 µL	Standard **C** (**0.5 ng/mL**)	C1/C2
50 µL	Standard **D** (**1 ng/mL**)	D1/D2
50 µL	Standard **E** (**2 ng/mL**)	E1/E2
50 µL	Standard **F** (**4 ng/mL**)	F1/F2
50 µL	Standard **G** (**8 ng/mL**)	G1/G2
50 µL	Control Serum **KS 1** (≥ 1:16 diluted)	H1/G2
50 µL	Control Serum **KS 2** (≥ 1:16 diluted)	A3/A4
50 µL	Sample (≥1:16 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation <u>with Shaking</u>: 1 h at 20°C - 25°C, <u>350 rpm</u>		
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	Enzyme Conjugate **EK**	In each well
Cover the wells with the sealing tape.		
Incubation <u>with Shaking</u>: 30 Minutes at 20°C - 25°C, <u>350 rpm</u>		
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: 15 Minutes in the Dark at 20°C - 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.		