IGFBP-2 - ELISA

Enzymeimmunoassay for the Determination of

Insulin-like Growth Factor Binding Protein-2

English

For Research Use Only. Not for Use in Diagnostic Procedures.

RUO Σ 96

REF **E05**

mediagnost®

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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 – partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ erä

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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/ Conținut suficient pentru x teste/ Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille

Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l´abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ Proteger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsätt inte för solljus/ Nie wystawiać na słońce/ Napfénytől távol tartandó/ Nevystavovať slnečnému svetlu/ Nevystavovat slunečnímu světlu/ Да се предпазва от слънчева светлина/ Kaitsta otsese päikesekiirguse eest/ Κρατήστε το μακριά από την ηλιακή ακτινοβολία/ Țineți departe de lumina soarelui/ Ne izpostavljajte sončni svetlobi/ suojaa auringonvalolta

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

Shaking/ Schütteln/ Mélanger/ Agitare/ Agitar/ Agitação/ Schudden/ Ryster/ Skaka/ Wstrząsanie/ Rázás/ Pretrepať/ Protřepat/ Разклащане/ Raputada/ Ανακινήστε/ Vibrare/ Stresite/ Sekoita

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

Ab CONJ	AK	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
DILU X	VP	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 διάλυμα X/ Diluați în tamponul X/ Razredčiti v pufru X/ laimennetaan x puskuriin
CAL X	A-E	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/ Kalibraattori X
Control	KS	Control Serum / Kontrollserum/ Contôle sérique/ Siero di controllo/ Suero de control/ Soro de Controlo/ controleserum/ Kontrolserum/ Kontrollserum/ Serum kontrolne/ Ellenőrző szérum/ Kontrolné sérum/ Kontrolní sérum/ Контролен серум/ Kontrollseerum/ Ορός ελέγχου/Ser de control/ Kontrolni serum/ Kontrolli seerumi
WASHBUF 20x	WP	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	S	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
H_2SO_4	SL	Stop Solution/ Stopp Lösung/ 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/ Soluție 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/ Aoperiți placa cu o bandă adezivă/ Prelepiti ploščo/ Peitä mikrotitrauslevy oheisella teipillä
MEASURE		Measure plate within 30 min at 450 nm (Referencefilter ≥590nm)/Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)./ Mesure lábsorbance en léspace de 30 min à450 nm avec ≥590nm 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 ≥590nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved nm (referencefilter ≥590nm)/ 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/Měřit 30 minut při 450 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 referință ≥ 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/ instrucțiuni internaționale 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 cavitățile necesare/ v vseh zahtevanih vdolbinah /kaikkiin tarvittaviin mikrotitrauslevyn syvennyyksiin

Read entire protocol before use!

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

** please ask for special package insert

PACKAGE INSERT ENGLISH

Mediagnost human **IGFBP-2 ELISA E05**

- Measures total IGFBP-2 concentration in serum, plasma and in other human body fluids
- single standards with **2; 10; 20; 40 and 80 ng/ml** are
- Total Incubation time only **1.75** hours
- 2 Control Sera are provided for quality control purposes according GLP
- ready-to-use Antibody Conjugate
- no sample extraction needed
- Microtiter plate seperately break apart

INTENDED USE

Measurement of human IGFBP-2 in human serum, EDTA-plasma, cerebrospinal fluid, breast milk, amniontic fluid, saliva and in cell culture medium.

IMPLICATIONS

The IGFBP-2 concentration is age-dependent in blood (3).

Normal values for healthy individuals (1.5 to > 70 years) were evaluated for this assay.

Supplementary parameter to IGFBP-3 in the diagnosis of growth disorders (IGFBP-2/IGFBP-3 ratio), IGFBP-2 is an inhibitor of growth hormone action (3,4).

Progression-dependent tumor marker in leukaemia (5), astrocystic CNS tumors (6,7), prostate- (8), suprarenal cortex-(9)-, hepatocellular (10) and other carcinomas.
Anti-aging parameter: IGFBP-2 as a marker of physiological functionality (20).

INTRODUCTION

Insulin-like growth factors (IGFs) regulate the proliferation, differentation, apoptose, cell adhesion and metabolism in various tissues and cell types. The IGF-I, which is produced mainly in liver under the influence of growth hormone (GH), regulates as hormone the linear growth of the bones and the process of sexual maturity, while IGF-II is mainly a growth factor of fetal tissue (11-13). The biological actions of IGF over the IGF-Type-I receptor are modulated variably through the IGF binding proteins (IGFBP-1 to-6) (14). IGFBP-2 is, after IGFBP-3, the second most frequent IGFBP in the human blood. IGFs, especially tumor typical pro-IGF-forms and hormones regulate the expression of IGFBP-2, GH effect is thereby inhibiting. At cellular level IGFBP-2 seems to stimulate the proliferation and dissemination of solid tumors via an IGF-independent mechanism (15,16).

PHYSIOLOGICAL MEANING

IGFBP-2 is a unglycosylated polypetide of 31.3 kDa, which forms binary IGF-complexes and shows no circadian rhythm in the circulation.The serum concentration of IGFBP-2 increases in fasting, after major surgery and after trauma, but the increasing of the concentration is most intensive in malignant diseases. The correlation of the IGFBP-2 level to the degree of progression is a striking feature in various tumor types as is the normalization of the IGFBP-serum levels after remission (5-10). During the GH-therapy, e.g. in short stature and in GH-abuse (doping) the IGFBP-2 level decreases. In Trisomy 18 IGFBP-2 in maternal serum is decreased and IGFBP-1 is increased; therefore the ratio IGFBP-2 /IGFBP-1 is a marker for this chromosome abnormality (17).

REAGENTS PROVIDED

1)	MTP	**Microtiter plate**, ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with antibody against anti-IGFBP-2 antibody packed in a laminate bag.
2)	CAL	**Standards A-E**, lyophilized: contain humanIGFBP-2: Standard values are between 2 - 80 ng/ml (2, 10, 20, 40, 80 ng/ml) IGFBP-2 and have to be reconstituted with **750 µl** Dilution Buffer **VP** each.
3)	DILU	**Dilution Buffer VP, 50 ml**, ready for use
4)	Control	**Control Serum KS1,** lyophilized: Contains human serum and has to be reconstituted with **100 µl Dilution Buffer VP**. The exact concentration of IGFBP-2 is given on the vial label.
5)	Control	**Control Sera KS2,** lyophilized: Contain human serum and has to be reconstituted with **100 µl Dilution Buffer VP**. The exact concentration of IGFBP-2 is given on the vial label.
6)	Ab CONJ	**Antibody POD-Conjugate AK, 12 ml**, ready for use: Contains a mix of biotinylated anti-human IGFBP-2 antibody and Horseradish peroxides conjugated streptavidin. Use 100 µl per well in the assay
7)	WASHBUF 20x	**Washing Buffer (WP), 50 ml**, 20 X concentrated solution. **Washing Buffer (WP)** has to be diluted 1:20 with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with A.dest. to 1000 ml). Please dilute only according to requirements. The diluted washing buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!
8)	SUBST	**Substrate (S), 12 ml**, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
9)	H_2SO_4	**Stopping Solution (SL), 12 ml**, ready for use, 0.2 M sulphuric acid, Caution acid!
10)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes and multichannel pipettes with disposable plastic tips
Distilled or deionized water for dilution of the Washing Buffer (WP)
Vortex-mixer
Microtiter plate shaker (350 rpm)
Microtiter plate washer (recommended)
Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
Polyethylen PE/Polypropylen PP tubes for dilution of samples

WARNINGS AND PRECAUTIONS

For in-vitro use only. For professional use only.
Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming. Do not mix reagents of different lots. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of human and/or animal origin. Source human serum for the **Control Sera KS1** and **KS2** provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

Following components contain < 0.01% 2-Methyl-4-isothiazolin-3-one solution as preservative : **AK, VP**

R34	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice

Following components contain < 0.01%(w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-one as preservative: **AK, VP, WP**

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice S28.1
S28.1	After contact with skin, wash immediately with plenty of water

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38 Irritating to eyes and skin
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1 After contact with skin, wash immediately with plenty of water
S36/37 Wear suitable protective clothing and gloves.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine. Store and Incubate in the dark.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

General first aid procedures:
Skin contact: Wash affected area thoroughly with water. Discard 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: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.
Do not eat, drink or smoke in these areas.
Never pipette the materials with the mouth.
Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

PRINCIPLE

The Mediagnost ELISA for IGFBP-2 E05 is a so-called Sandwich-Assay using two specific and high affinity antibodies. The IGFBP-2 in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-IGFBP-2-Antibody binds in turn to the immobilised IGFBP-2. The second antibody is biotinylated and will be applied in a mixture with a Streptavidin-Peroxidase-Enzyme Conjugate. In the closing substrate reaction the turn of the colour will be catalysed quantitatively depending on the IGFBP-2-level of the samples.

SPECIMEN

Serum and plasma samples as well as cell culture medium, breast milk, amniotic fluid, cerebrospinal fluid and saliva are applicable.

The blood sample for serum preparation should be gained according to standardized venipucture procedure. Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.

Blood samples may be taken at any time of the day.

Storage of the samples

Storage at RT max. 2 days

Storage at –20°C max. 2 years

Are not allowed to have more than 10 freeze/thaw cycles.

Sample Preparation

Samples have to be diluted 1:10-30-fold with Dilution Buffer (VP).
A standard dilution of **1:21** is rsuggested**.**

Suggestion for dilution protocol:
Mix 15 µl serum manually or with the aid of a dilutor with 300 µl Dilution Buffer VP (1:21). Use 2 x 100 µl of this dilution in the assay or pipette 100 µl buffer in wells and add 5 µl serum.
IGFBP-2 concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatant (s.Table 5).

TECHNICAL NOTES

The assay has to be conducted strictly according the test protocol herein.

Reagents with different lot numbers cannot be mixed. The microtiter plate and reagents are stable until the indicated expiry if stored unopened and protected from sunlight at 2 – 8°C.

The shelf life of the components after opening is not affected, if used appropriately.

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.

Incubation at room temperature means: Incubation at 20-25°C

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 become 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.

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 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 dynamicly 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.

Standards and Controls

For the reconstitution of the lyophilised components (Standards A - E and Control Sera KS1 &KS2) the kit Dilution Buffer VP has to be used. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam!) with a Vortex mixer.

The reconstituted standard and controls can be stored for 2 months at –20°C. Repeated freeze/thaw cycles have to be avoided.

Washing Buffer

The required volume of washing buffer is prepared by 1:20 dilution of the provided 20fold concentrate with deionised water. The diluted washing buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

Substrate

The Substrate Solution S, stabilized H_2O_2- Tetramethylbenzidine, is photosensitive– Storage and Incubation in the dark.

Microtiter plate

Unused microtiter plate stripes have to be stored airtight together with the desiccant bag at 2-8°C. The labelled expiry is not influenced in case of proper storage.

ASSAY PROCEDURE

All determinations (Standards, 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 Standards, Control Sera and the samples should be pipette as fast as possible (e.g., <15 minutes).

All incubations have to be conducted at room temperature (20-25°C)!

To avoid distortions due to differences in incubation times, Antibody-POD-Conjugate AK as well as the following Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. Stop Solution SL should be added to the plate in the same order as the Substrate Solution.

1) Add **100 µl** Dilution Buffer VP to the first wells (blank). Subsequently, add **100 µl** of each **Standard** or diluted **Control (KS1&KS2)** or diluted **Sample** to the following wells.

2) Cover the wells with sealing tape and incubate the plate for **1 hour** shaking with **350 rpm**.

3) After incubation aspirate the contents of the wells into a disinfectant (possible theoretically risk of infection!) and wash the wells **5 times** with **300 µl** of **Washing Buffer WP** / well respectively. The washing buffer WP should incubate at least for 15 seconds/cycle.

4) Pipette **100 µl** of the **Antibody-POD-Conjugate AK** in each well and incubate **30 minutes** shaking with **350 rpm**.

5) After incubation wash the wells 5 times with Washing Buffer as described in step 3.

6) Pipette **100 µl of the Substrate (S)** in each well.

7) Incubate the plate for **15 minutes in the dark at room temperature (20 - 25°C)**.

8) Stop the reaction by adding **100 µl of Stopping Solution (SL)**.

9) Measure the colour reaction within 30 minutes at **450 nm** (**reference filter ≥ 590 nm**).

CALCULATION OF RESULTS

Establishing the Standard Curve

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, these of standard E should be above 1.0.

Samples, which yield higher absorbance values than Standard E are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

Standards are provided in the following IGFBP-2 concentrations

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	2	10	20	40	80

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 values.

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. **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-2 concentration of the diluted sample or the diluted control sera KS1&2 in ng/ml is calculated in this way, the IGFBP-2 concentration of the **undiluted sample** and of KS1 & KS2 is calculated **by multiplication** with the respective dilution factor.

The exemplary shown standard curve in Fig.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 the IGFBP-2 concentration of undiluted sample:

Measured extinction of your sample	0.37
Measured extinction of the blank	0.06

Your **measurement programm** will calculate the IGFBP-2 concentration of the diluted sample automatically by using the difference (0.31) of sample and blank for the calculation. 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 programm to calculate the IGFBP-2 concentration in the sample:

$0.31 = -0.0012048 + 0.039581x + 5.1788 \cdot 10^{-0,005} \cdot x^2 - 1.8929x\ 10^{-0,06} \cdot x^3$

$7.93 = x$

if the dilution factor (**1:21**) is taken into account the IGFBP-2 concentration of the undiluted sample is $7.93 \cdot 21 = 166.55$ ng/ml

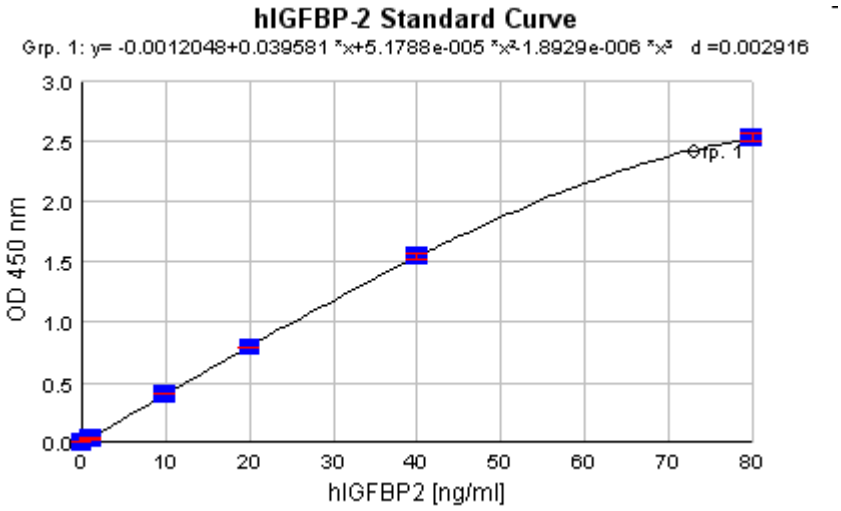


Fig. 1: Exemplary Standard Curve with a polynomial 3rd degree as curve fit.

PERFORMANCE CHARACTERISTICS

Standards

The Standards of the ELISA E05 are prepared from **human IGFBP-2** in concentrations of 2, 10, 20, 40 and 80 ng/ml.

Sensitivity

The analytical sensitivity of the assay yields 0.2 ng/ml (2x SD of zero standards)

Specificity

This assay is specific for human IGFBP-2, only low degree of cross reactions was found with dog, horse, donkey, cat and goat. No cross-reactivity was with pig, bovine, rabbit, mouse, chicken, rat, guinea pig, sheep.

There was no cross reactivity with IGFBP-1 and IGFBP-3.

Interference

Interference of bilirubin and triglycerides was tested by adding different amounts of these substances to human serum containing IGFBP-2. For comparison the same amount of buffer without any substance was also added to the serum. Table 1 demonstrates that neither bilirubin nor triglycerides exert any influence on the measurement of IGFBP-2 in human serum.

Table 1: Interference

Bilirubin			**Triglycerides**	
[µg/ml]	% of control		[mg/ml]	% of control
25	95.07		12.5	100.79
50	92.80		25	101.01
100	93.83		50	103.65
200	88.15		100	101.34

Recovery

Recombinant IGFBP-2 was added in three different concentrations to human serum. The IGFBP-2 concentration was measured and the mean relative recovery in comparison to buffer was 108%. Some exemplary data are shown in table 2.

Table 2: Recovery of recombinant human IGFBP-2 in Serum

IGFBP-2 [ng/ml]	+1000 ng/ml	+500 ng/ml	+100 ng/ml	Mean [ng/ml]
% Recovery	100,00	112,00	114,00	108,67

Reproducibility and Precision

The inter- and intra assay coefficients of variability are below 10%. Exemplary determinations are shown in table 3 and 4.

Table 3: Interassay-Variation

Sample1 (ng/ml)	137	159	152
Sample 2 (ng/ml)	672	697	688
Sample 3 (ng/ml)	928	929	956

Table 4: Intra-Assay-Variation

Sample 1 ng/ml	322	375	298	305	318	311	320	325	302	301	305	317
Sample 2 ng/ml	612	609	616	648	594	597	620	613	617	611	636	698

Table 5: Linearity of the sample dilution:

Dilution	Serum 1 (ng/ml)	Serum 2 (ng/ml)	Cerebrospinal fluid (ng/ml)	Amniotic fluid (ng/ml)
1:10	938	582	426	Not determined
1:20	1061	673	428	460
1:40	1055	719	379	483
1:80	1004	691	318	431
1:160	952	668	426	415

REFERENCE VALUES

The IGFBP-2 concentration in serum is depended on age (Table 8) and on Body Mass Index (BMI; Table 7). For data collection of these reference values IGFBP-2 levels were determined in serum of over 400 normal children and adults (see table 8 figure 2); (3).

Please see the expected values of IGFBP-2 levels in other human body fluids than serum and in cell culture medium in the table 6.

LIMITATION

Deviation from the reference range can be expected especially in hypothyroidism, after major surgery, in polytrauma, in Diabetes mellitus (due to insulin therapy), in fasting and in malignant diseases.

APPENDIX

Table 6 : Expected values of IGFBP-2 in body fluids of human origin and in cell culture supernatants:

Sample	Expected Value [ng/ml]
Serum	[100 - 1000]
Cerebro-spinal fluid	[100 - 300]
Amniotic fluid	[200 - 10000]
Seminal plasma	[5000 - 15000]
Breast milk	[1500-3000]
Cell culture supernatants	[5 - 300]

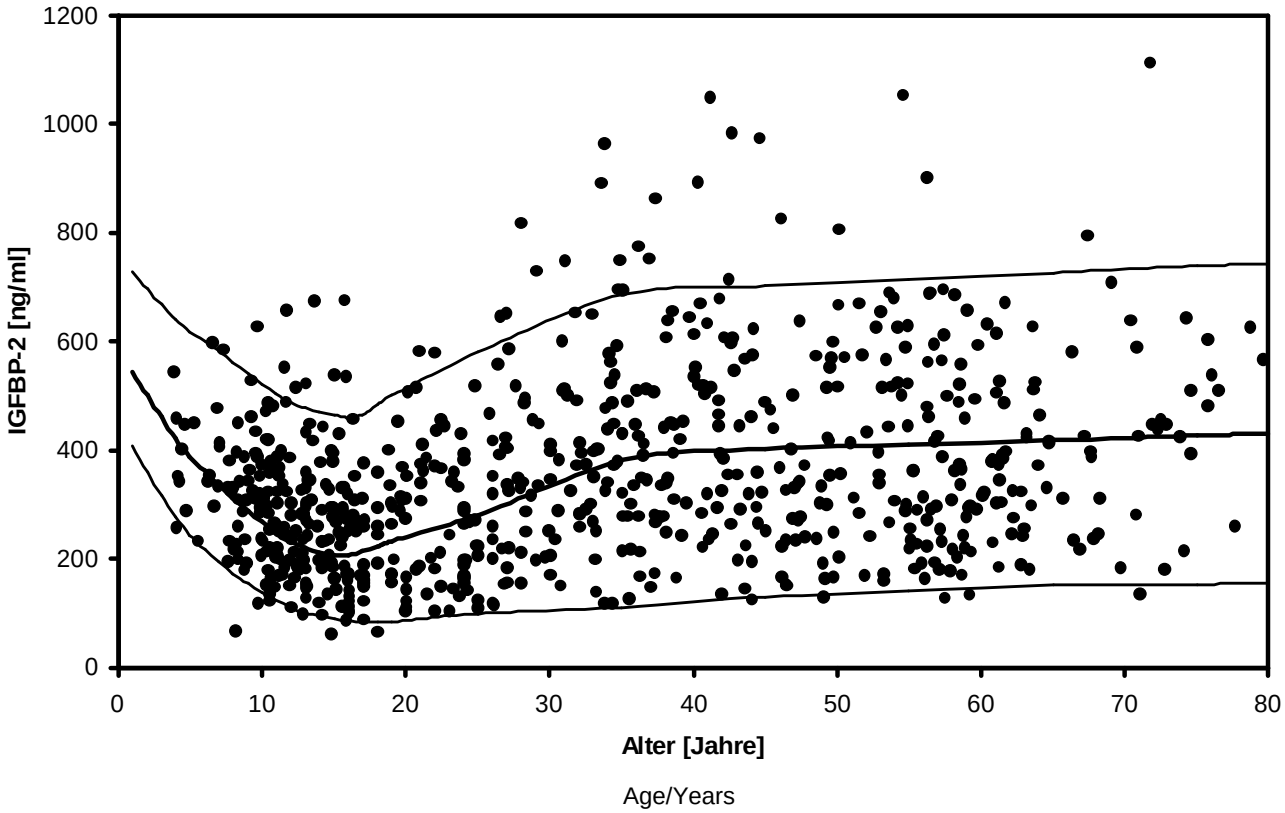
Table 7: BMI dependent reference values, adults between 20 and 80 years

BMI		**mean**	SA	**percentiles**		
[kg/m^2]	N	**IGFBP-2 [ng/ml]**	SD	5.	50.	95.
15	12	612	110	431	612	793
17,5	14	568	126	361	568	775
20	76	509	144	271	509	746
22,5	124	449	162	182	449	716
25	101	398	165	127	398	670
27,5	52	348	147	106	348	590
30	25	315	118	120	315	510
32,5	15	282	90	135	282	430
35	4	251	80	119	251	383
37,5	4	220	71	104	220	336

Table 8: IGFBP-2 serum levels (in ng/ml) of > 400 healthy individuals. The normal range is given by the 5., 50. and 95. percentile for age classes.

Age-dependent normal range of serum IGFBP-2

age (years)	5. Percentile (ng/ml)	50. Percentile (ng/ml)	95. Percentile (ng/ml)
1	408	545	728
2	359	500	696
3	317	460	668
4	277	421	640
5	243	388	617
6	217	361	602
7	194	336	583
8	173	312	562
9	154	289	542
10	138	268	522
11	123	249	503
12	111	232	486
13	101	219	477
14	94	212	470
15	89	207	465
16	86	207	460
17	84	214	466
18	84	223	483
19	84	232	500
25	99	280	580
35	110	381	686
45	130	403	702
55	140	410	715
65	151	418	727
75	153	427	740
80	156	430	744



Von Dr. R. Schweizer, Tübingen, zusammengestellt (3). Assembled by Dr. R. Schweizer, Tübingen,

Fig. 2: IGFBP-2 serum levels (in ng/ml) of > 400 healthy individuals. The normal range is given by the 5th, 50th and 95th percentile.

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SUMMARY – MEDIAGNOST IGFBP-2 ELISA E05

Reagent:	**Reconstitution:**	**dilution:**
Standards A-E	in **750 µl** Dilution Buffer **VP**	
Control Serum KS1	in **100 µl** Dilution Buffer **VP**	**1:21 with** Dilution Buffer **VP**
Control Serum KS2	in **100 µl** Dilution Buffer **VP**	**1:21 with** Dilution Buffer **VP**
Washing Buffer WP		**1:20** with **Aqua. dest.** (e.g., add the complete contents of the flask **(50 ml)** into a graduated flask and fill with **A.dest.** to 1000 ml).
Sample Dilution: Serum samples should be diluted prior to measurement 1:10-30-fold with **Dilution Buffer VP** depending on the expected values. In general a dilution of 1:21 is appropriate. Use 2 x 100 µl of this dilution in the assay		

Assay Procedure for Double Determination

Pipette	**Reagents**	**Position**
100 µl	Dilution Buffer **VP**	A1/2
100 µl	Standard **A** (**2 ng/ml**)	B1/2
100 µl	Standard **B** (**10 ng/ml**)	C1/2
100 µl	Standard **C** (**20 ng/ml**)	D1/2
100 µl	Standard **D** (**40 ng/ml**)	E1/2
100 µl	Standard **E** (**80 ng/ml**)	F1/2
100 µl	Controll Serum **KS1**	G1/2
100 µl	Controll Serum **KS2**	H1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl Wash Buffer WP**	each well
100 µl	**Antibody-POD-Conjugate AK**	each well
Incubation: 30 min at RT, 350 rpm		
5x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl Wash Buffer WP**	each well
100 µl	Substrate Solution **S**	each well
Incubation: 15 min in the Dark at RT		
100 µl	Stopping Solution **SL**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

REF E05 INTERNATIONAL TEST DESCRIPTION

CAL **A-E**	**A -E**	**Rec in** 750 µl **VP**	
Control	**KS1 & KS2**	**Rec in** 100 µl **VP**	**1:21** **DILU** **VP**
WASHBUF **20x**	**WP**		**1:20** **DILU** **A. dest.**

SPE	**1:21** **DILU** **VP**
°C **20-25 °C**	

100 µl	**VP**	A1/2
100 µl	**CAL** **A** **A** **(2 ng/ml)**	B1/2
100 µl	**CAL** **B** **B** **(10 ng/ml)**	C1/2
100 µl	**CAL** **C** **C** **(20 ng/ml)**	D1/2
100 µl	**CAL** **D** **D** **(40 ng/ml)**	E1/2
100 µl	**CAL** **E** **E** **(80 ng/ml)**	F1/2
100 µl	**CONTROL** **KS1**	G1/2
100 µl	**CONTROL** **KS2**	H1/2
100 µl	**SPE**	
TAPE		

1 h **°C** **20-25** ↔ **350 rpm**

5x 300 µl	**5x** **WASHBUF** **WP**
100 µl	**AbCONJ** **AK**
TAPE	

0.5 h **°C** **20-25** ↔ **350 rpm**

5x 300 µl	**5x** **WASHBUF** **WP**
100 µl	**SUBST** **TMB** **S**

15 min **°C** **20-25**

100 µl	**H_2SO_4** **SL**
MEASURE	