IGFBP-2 Elisa

KAPME05

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History

Summary of change :

Previous Version : 200224/1	**Current Version :** 210527
INTENDED USE Measurement of human IGFBP-2 in human serum, EDTA-plasma, cerebrospinal fluid, breast milk, amniontic fluid, saliva and in cell culture medium.	**INTENDED USE** Measurement of human IGFBP-2 in human serum and EDTA-plasma.
Sample Preparation and Storage 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. The samples should be stored without anticoagulation reagents. 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 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 and Storage Serum and plasma samples are applicable. The blood sample for serum preparation should be gained according to standardized venipucture procedure. The samples should be stored without anticoagulation reagents. 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 and should be stored in fermly closed sample vials. Haemolytic reactions are to be avoided. Storage at RT max. 24 hours Storage at –20°C max. 2 years Are not allowed to have more than 10 freeze/thaw cycles.
Materials not Provided NA	Materials not provided Addition of "Polyethylene PE/Polypropylene PP tubes for dilution of samples.
Technical hints No information regarding the washing and shaking steps.	Technical hints Information added
PROCEDURE wash the wells 3 times with 250 µl of Washing Buffer / well respectively.	Procedure wash the wells 5 times with 300 µl of Washing Buffer / well respectively.
No information regarding the quality control	Information added
PERFORMANCE CHARACTERISTICS ***Sensitivity*** The analytical sensitivity of the assay yields 0.2 ng/ml (2x SD of zero calibrator)	**PERFORMANCE CHARACTERISTICS** Sensitivity Sensitivity was assed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the E05 is 0.2 ng/mL.
PERFORMANCE CHARACTERISTICS **Table 5**: Linearity of the sample dilution: Dilution test performed on Two serum samples used as matrix, Cerebrospinal fluid and Amniotic fluid.	**PERFORMANCE CHARACTERISTICS** **Table 5**: Linearity of the sample dilution: Dilution test performed on Two serum samples used as matrix
Interference No data for Hemoglobin interference	**Interference** Data for Hemoglobin interference added
No information regarding Instructions for use for scientific application	Information added

IGFBP-2-ELISA

Enzyme Immunoassay for the Quantitative Determination of Insulin-like Growth Factor Binding Protein-2

KAPME05

IN VITRO DIAGNOSTIC USE

en

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INTENDED USE

Measurement of human IGFBP-2 in human serum and EDTA-plasma.

CLINICAL 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).

MATERIALS

Materials Provided

1)  **Microtiter plate**, ready for use: **Microtiter plate** with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-IGFBP-2 antibody and packed in a laminate bag.

2) CAL | N — **Calibrators 1-5,** lyophilized: contain recombinant IGFBP-2: Calibrator 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** each.

3) CONTROL | N — **2 Controls,** lyophilized: Contains human serum and has to be reconstituted with **100 µl** Dilution Buffer. The exact concentration of IGFBP-2 is given on the vial label.

4) DIL | BUF — **Dilution Buffer**, 50 ml, ready for use.

5) Ab | BIOT / SAV | HRP — **Antibody-Conjugate,** 12 ml, ready for use, contains a mixture of rabbit-anti-hIGFBP-2-antibody biotinylated + streptavidin horseradish peroxidase conjugate ready for use. Use 100 µl/well in the assay.

6) WASH | SOLN | CONC — **Washing Buffer**, 50 ml, 20-fold concentrated: The **Washing Buffer** has to be diluted 1:20 with distilled water 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 !

7) CHROM | TMB **TMB-Substrate Solution** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised Tetramethylbencidine.

8) STOP | SOLN **Stopping Solution**, 0.2 M sulphuric acid, 12 ml, ready for use. *Caution Acid !*

9) Sealing tape for covering of the microtiter plate, 2 x

Materials not Provided

- Precision pipettes (100 and 200µl) Micropipettes and multichannel pipettes with disposable plastic tips
- Distilled or Deionized water for dilution of the washing buffer
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Micropipettes and multichannel pipettes with disposable plastic tips
- Vortex-mixer
- Plate washer and plate shaker (≥ 350 rpm) (recommended)
- Calibrated Microplate reader ("ELISA-Reader") with filter for 450/620nm wavelength
- Timer (120 min. range)

WARNING AND PRECAUTIONS

The kit should not be used beyond the expiration date on the kit label.
For In Vitro Diagnostic Use only. For Professional use only.
The DIAsource kit is suitable only for in vitro diagnostics 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. DIAsource 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 obviously 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. Material Safety Data Sheet is available on request.

Human Serum
Following components contain human serum: Controls.
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 Calibrators 1-5, Antibody-Conjugate, Dilution Buffer, Washing Buffer
Contain as preservative a mixture of 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.

TMB-Substrate Solution
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
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.

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.

METHODOLOGY

Principle

The DIAsource IGFBP-2 Elisa kit 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.
It recognizes IGFBP-2 quantitatively and is not influenced by increased IGF-I or IGF-II values. Related molecules such as IGFBP-3 do not show any cross-reactions in the test.

Sample Preparation and Storage

Serum and plasma EDTA samples are applicable.
The blood sample for serum preparation should be gained according to standardized venipucture procedure. The samples should be stored without anticoagulation reagents. Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.
Whole blood should ideally be separated after about 2 hours and processed after 12 hours at the latest and stored at -20 ° C until the measurement.
Blood samples may be taken at any time of the day and should be stored in fermly closed sample vials. Haemolytic reactions are to be avoided.

Storage at RT max. 24 hours
Storage at –20°C max. 2 years
Are not allowed to have more than 10 freeze/thaw cycles.

Triglycerides bilirubin and hemoglobin in the sample do not interfere up to a concentration of 100 mg/mL, 200 µg/mL and 1 mg/mL respectively. The use of hemolytic, lipemic or icteric samples should nevertheless be validated beforehand by the user.

Sample dilution
Dilution: 1:21 with Dilution Buffer
Example: 15 µL sample are added to 300 µL Dilution Buffer (dilution factor 21). After mixing this solution, 2 x 100 µL are used in the assay.

The serum samples must be diluted 1:10 – 1:30 with Dilution Buffer prior to measurement, depending on the expected IGFBP-2 values. In general, a dilution of 1:21 is suitable (and thus the minimum required sample volume for a duplicate determination is 15 µL serum). An extraction step is not required.

Technical hints

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

Reagents with different lot numbers cannot be mixed.

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. Store the unused seal stripes of the microtiterplate together with the desiccant at 2-8°C.

Calibrators and Controls
For the reconstitution of the lyophilised components (calibrators 1-5 and Control Sera), the kit Dilution Buffer 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 calibrator and controls can be stored for 4 weeks 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.
After reconstitution dilute the Controls with the Dilution Buffer in the same ratio (1:21) as the sample.

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.

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!

Incubation at room temperature means: Incubation at 20 - 25°C. The TMB-Substrate Solution 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 approx. 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 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.

Manual washing should be used. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. Decant contents into a biohazard bin, then blot plate on absorbent tissue. Wash the plate by adding 300µL Washing Buffer WB/well, then decant and blot on absorbent tissue. Repeat this step 4 more times for total of 5 washes.

ASSAY PROCEDURE

All determinations (Calibrators, Control Sera and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the **Calibrators 1-5, Controls and samples** should be pipetted as fast as possible (e.g., 15 Minutes). To avoid distortions due to differences in incubation times, antibody-Conjugate and the TMB-**Substrate solution** should be added to the plate in the same order and the same time interval as the samples. **Stopping Solution** should be added to the plate in the same order as the **TMB-Substrate Solution.**

1) After reconstitution dilute the Controls with the Dilution Buffer in the same ratio (1:21) as the sample.
2) Add **100 µl** Dilution Buffer to the first wells (blank). Subsequently, add **100µl** of each Calibrator or diluted **Control (1 & 2)** or diluted **Sample** to the following wells.
3) Cover the wells with sealing tape and incubate the plate for **1 hour** shaking at room temperature at **350 rpm**.
4) After incubation decant the contents of the wells into a disinfectant (risk of infection!) and wash the wells 5 times with **300 µl** of **Washing Buffer** / well respectively.
5) Following the last washing step pipette **100µl** of the **Antibody-Conjugate** in each well. Cover the wells with the sealing tape and incubate the plate for **30 Minutes** shaking at **room temperature at 350 rpm**.
6) After incubation wash the wells 5 times with **Washing Buffer** as described above.
7) Pipette **100 µl** of the **TMB-substrate** solution in each well.
8) Incubate the plate for **15 minutes** in the dark at **room temperature**.
9) Stop the reaction by adding **100 µl** of **Stopping Solution** to all the wells.
10) Measure the absorbance within **30 minutes** at **450 nm** with ≥ 590 nm as reference wavelength.

QUALITY CONTROL
Good laboratory practice requires that controls are included in each assay. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws.

Quality criteria
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 Calibrator 5 should be above 1.00. Samples, which yield higher absorbance values than Calibrator 5, should be re-tested with a higher dilution.

EVALUATION OF RESULTS

Establishing the Calibration Curve:
The calibrators provided contain the following concentrations of IGFBP-2:

Calibrators	**1**	**2**	**3**	**4**	**5**
ng/ml	2	10	20	40	80

1) Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).

2) Subtract the mean absorbance (MA) of the blank from the mean absorbance of all other values.

3) Plot the calibrators concentrations 1-5 on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis. Read the concentration of each control and sample by interpolation on the calibration curve.

4) Recommendation: Calculation of the calibration 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 four parametric logistic (4-PL) curve fit should be used for recalculation of IGFBP-2 concentrations.

5) The IGFBP-2 concentration of the diluted sample or the diluted control sera 1&2 in ng/ml is calculated in this way, the IGFBP-2 concentration of the **undiluted sample** and of 1 & 2 is calculated **by multiplication** with the respective dilution factor.

Example of a typical calibration curve

The exemplary shown calibration curve in Fig.1 **cannot be used** for calculation of your test results. You have to establish a calibration 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 program** 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 program to calculate the IGFBP-2 concentration in the sample:

$0.31 = -0.0012048 + 0.039581x + 5.1788 \cdot 10^{-0.005} \cdot x^2 - 1.8929x\ 10^{-0.006} \cdot x^3$
$7.93 = x$
if the dilution factor (**1:21**) is taken into account the IGFBP-2 concentration of the undiluted sample is 7.93 x 21= 166.55 ng/ml

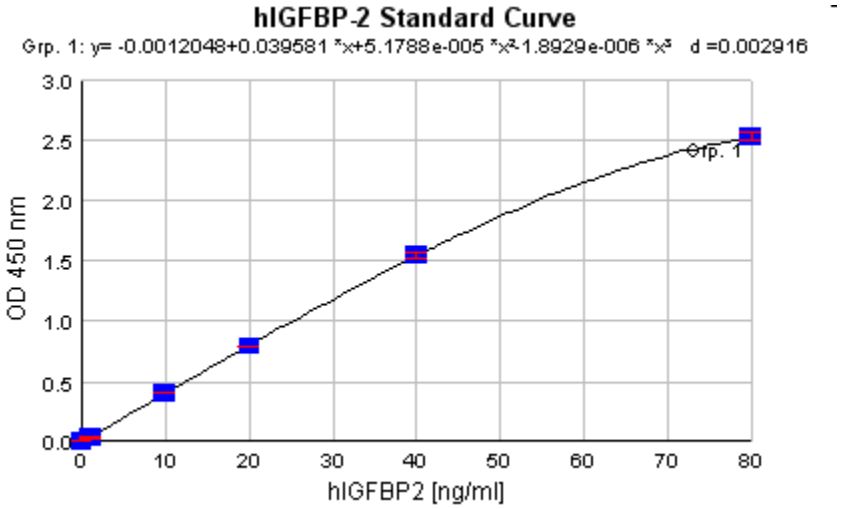


Fig. 1: Exemplary Calibration Curve with a polynomial 3rd degree as curve fit.

INTERPRETATION OF RESULTS

The test results should not be the only base for therapeutic decisions. The results should be interpreted in regard to anamnesis, further clinical observations and results of other diagnostic investigations. Further, it is recommended to establish reference and cut-off values corresponding to the relevant group of patients for each laboratory.

PERFORMANCE CHARACTERISTICS

Sensitivity
Sensitivity was assed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the KAPME05 is 0.2 ng/mL.

Specificity
The specificity of this ELISA was investigated. Neither 100 ng/mL IGFBP-1 nor 20 µg/mL IGFBP-3 containing samples resulted in a signal significantly different of the blank.

Interference
Interference of bilirubin, triglycerides and hemoglobin 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**		**Hemoglobin**	
[µg/ml]	% of control	[mg/ml]	% of control	[mg/mL]	% of control
25	95.07	12.5	100.79	0.125	99
50	92.80	25	101.01	0.25	105
100	93.83	50	103.65	0.5	100
200	88.15	100	101.34	1	100

Recovery

Recombinant IGFBP-2 was added in three different concentration 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 : Inter-Assay-Variation

Sample1 (ng/ml)	137	159	152	8% CV
Sample 2 (ng/ml)	672	697	688	2% CV
Sample 3 (ng/ml)	928	929	956	2% CV

Table 4: Intra-Assay-Variation

Sample 1 ng/ml	322	375	298	305	318	311	320	325	302	301	305	317	6.48% CV
Sample 2 ng/ml	612	609	616	648	594	597	620	613	617	611	636	698	4.49% CV

Linearity

The test can be diluted over a very wide range. The linearity of serum dilutions is shown in table 5.

Table 5: Linearity of the sample dilution:

Dilution	Serum 1 (ng/ml)	Serum 2 (ng/ml)
1:10	938	582
1:20	1061	673
1:40	1055	719
1:80	1004	691
1:160	952	668

EXPECTED VALUES

The IGFBP-2 concentration in serum is depended on age (Table 6) 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 7 figure 2); (3).

Table 6: BMI dependent reference values, adults between 20 and 80 years

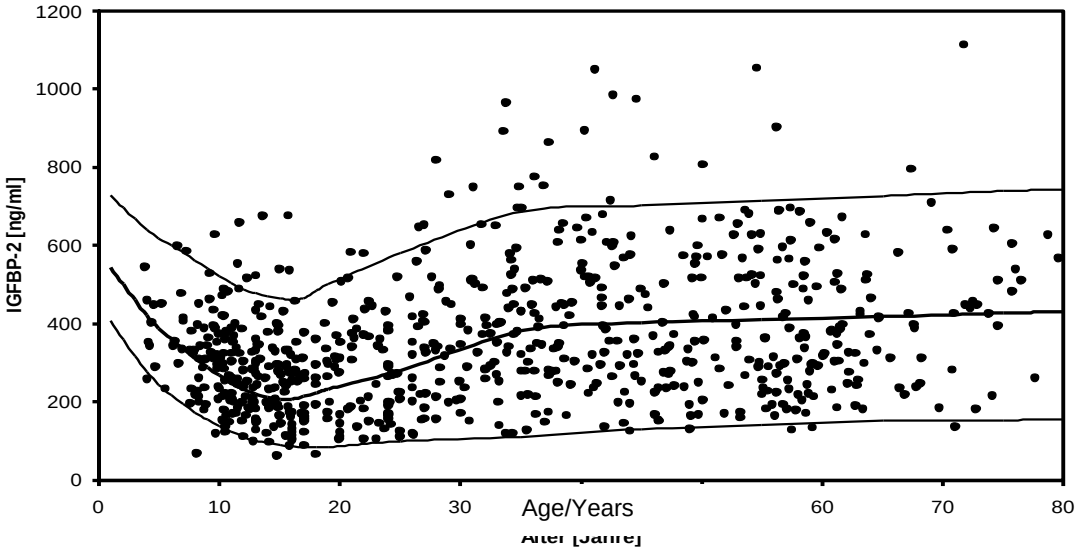
BMI		**IGFBP-2 [ng/ml]**		**percentiles**		
[kg/m^2]	N	mean	SD	5th	50th	95th
15	12	612	110	431	612	793
17,5	14	568	126	361	568	775
20	76	509	144	271	509	746
22,5	124	449	162	182	449	716
25	101 398	165	127	398	670	
27,5	52 348	147	106	348	590	
30	25 315	118	120	315	510	
32,5	15 282	90	135	282	430	
35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

Table 7 : 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



Assembled by Dr. R. Schweizer, Tübingen, Germany

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

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.

The DIAsource IGFBP-2 ELISA is based on mono- and polyclonal antibodies. Generally the result of any immunological test system can be influenced by heterophilic antibodies, anti-species antibodies or rheumatic factors. The assay design reduces these potential influences to a minimum but they cannot be excluded in any case.

Instructions for use for scientific application

SCIENTIFIC APPLICATION

IGFBP-2 is present in different concentrations in various body fluids and in conditioned cell culture media of many cell lines.

Samples suitable for scientific application

Cerebrospinal fluid, breast milk, amniotic fluid, saliva and in cell culture medium.

Table 8 : Linearity of the sample dilution.

Dilution	Cerebrospinal fluid (ng/mL)	Amniotic fluid (ng/mL)
1:10	426	Not determined
1:20	428	460
1:40	379	483
1:80	318	431
1:160	426	415

Table 9 : Expected values of IGFBP-2 in body fluids of human origin and in cell culture supernatants:

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

Table 10: Results of saliva-sample matrix tests:

Recombinant IGFBP-2: 38,2 [ng/mL] % Recovery		
Saliva + DIL	2.02	n.a.
Saliva + rec. IGFBP-2	35.7	89
Saliva + rec. IGFBP-2	37.36	93
Saliva + rec. IGFBP-2	35.67	89
Saliva + rec. IGFBP-2	36.17	90

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

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SUMMARY OF THE ASSAY

Reagent:	**Reconstitution:**	**dilution:**
Calibrators 1-5	in **750 µl** Dilution Buffer	
Controls Serum	in **100 µl** Dilution Buffer	**1:21 with** Dilution Buffer
Washing Buffer		**1:20** with **Distilled water**
Dilute samples 1:21 in Dilution Buffer, mix immediately, Use **100 µL** for each well in the assay.		
Before assay procedure bring all reagents to room temperature +20-25°C.		

Assay Procedure for Double Determination

Pipette	**Reagents**	**Position**
100 µl	Dilution Buffer	A1/2
100 µl	**Calibrator 1** (**2 ng/ml**)	B1/2
100 µl	**Calibrator 2** (**10 ng/ml**)	C1/2
100 µl	**Calibrator 3** (**20 ng/ml**)	D1/2
100 µl	**Calibrator 4** (**40 ng/ml**)	E1/2
100 µl	**Calibrator 5** (**80 ng/ml**)	F1/2
100 µl	Control Serum 1 (1:21 diluted)	G1/2
100 µl	Control Serum 2 (1:21 diluted)	H1/2
100 µl	Sample dilution (1:21 diluted)	following wells
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, 350 rpm		
5x 300 µl	Decant the contents of the wells and wash 5**x** with 300 **µl Wash Buffer**	each well
100 µl	**Antibody-Conjugate**	each well
Cover the wells with the sealing tape.		
Incubation: 30 min at RT, 350 rpm		
5 x 300 µl	Decant the contents of the wells and wash 5 **x** with 300 **µl Wash Buffer**	each well
100 µl	TMB-Substrate Solution	each well
Incubation: 15 min in the Dark at RT		
100 µl	Stopping Solution	each well
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

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