Human IGFBP-2 ELISA

(Insulin-Like Growth-Factor Binding Protein-2)

Cat. No.: RMEE05R

TECHNICAL FEATURES

- 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
- Microtiterplate 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.

CLINICAL IMPLICATIONS

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

INTRODUCTION

Insulin-like growth factors (IGFs) regulate the proliferation, differentation, apoptose, cell adhesion and metabolism in various tissues and cell types. The IGF-1, 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-2 is mainly a growth factor of fetal tissue (11-13). The biological actions of IGF over the IGF-Type-1 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). The IGFBP-2 concentration is age-dependent in blood (3).

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 (100 and 200 µl) Micropipettes and multichannel pipettes with disposable plastic tips
Distilled or Deionized water for dilution of the Washing Buffer (WP)
Vortex-mixer
Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)
Timer (120 min. range)
Reservoirs (disposable)
Microtiterplate Shaker (350 rpm)
Microtiter Plate washer (recommended)
Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620 nm (or ≥590 nm)
Foil welding device for laminate bags (recommended)
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 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. Cleand contaminated areas and equipment with a suitable detergent.

PRINCIPLE

The ELISA for IGFBP-2 RMEE05 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. A special external sample preparation prior to assay is not required.

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case of a longer time period between the sampling and measurement, store the undiluted samples at -20°C or below in tightly closable plastic tubes. Avoid repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although IGFBP-3 levels were found to be unaffected by few cycles (5x) in our experiments.
The high sensitivity of the assays allows IGFBP-2 determinations in small sample volumes, which is limited by pipetting accuracy rather than the amount of IGFBP-2.
In most determinations (e.g. Serum- or Plasma samples and no extreme values expected) the dilution of **1:10 to 1:30 with**

Sample Buffer PP is suitable. The IGFBP-2 concentrations might be completely different in body fluids of human origin other than serum or in cell culture supernatants. A standard **dilution of 1:21** is suggested.
Suggestion for dilution protocol:
Mix **15 µl sample** 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

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.

TECHNICAL RECOMMENDATIONS

The assay has to be conducted strictly according the test protocol herein.
Reagents with different lot numbers cannot be mixed. The microtiterplate and reagents are stable until the indicated expiry, if stored unopened and protected from sunlight at 2 – 8°C.
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: 20-25°C
The incubation steps should be performed at mean rotation frequency of a particularly suitable microtitre 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 microtitre 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 microtitre 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 Sample Buffer PP 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 should result) with a Vortex mixer.

The reconstituted standards and controls can be stored for 3 months at –20°C. Repeated freeze/thaw cycles have to be avoided. When using the standards anew, please thaw them rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay.

Washing Buffer
The required volume of washing buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for max. 4 weeks at 2-8°C.

Substrate Solution
The **Substrate Solution S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

Microtiterplate
Store the once unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at 2-8°C use in the frame provided. The labelled expiry is not influenced in case of proper storage.

WARNINGS AND PRECAUTIONS

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Temperature WILL affect the absorbance readings of the assay. However, values for the samples will not be affected.

Do not use expired reagents.

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.

Caution: This kit contains material of human and/or animal origin.

Human Serum

Contained in following components: **Control Serum KS1 and KS2.**

The sources of human sera were 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) antibodies. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and specimens should be treated as potentially infectious.

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

2-Methyl-4-Isothiazolin-3-one

contained in following components: **AK, VP, PP**

< 0.01% 2-Methyl-4-isothiazolin-3-one Solution

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

5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one

contained in following components: **AK, VP, WP, PP**

< 0.01% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one Solution

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	After contact with skin, wash immediately with plenty of water

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine.

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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.

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 450nm (reference filter 620nm).

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

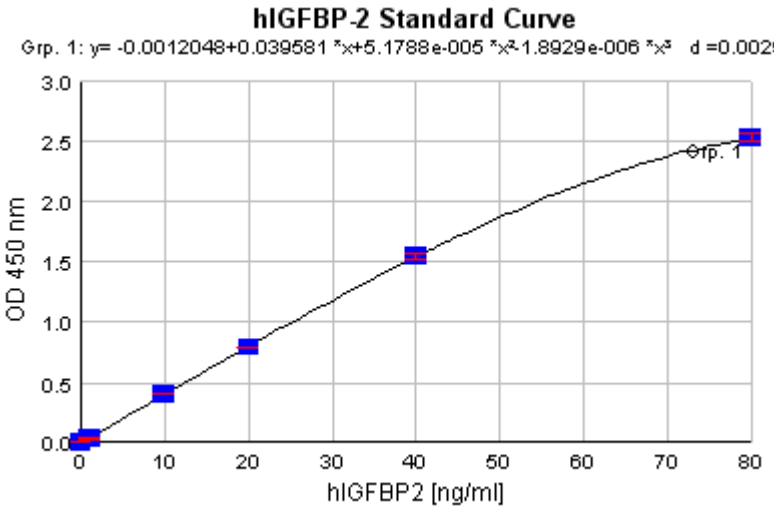


Fig. 1: Exemplary Standard Curve with a polynomial 3rd degree as curve fit.

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.

APPENDIX

Table 6 : Exemplary results of IGFBP-2 measurements in body fluids of human origin and in cell culture supernatants:

Sample	**Concentration Range [ng/ml]**
Serum/ serum	[100 - 1000]
Liquor/ cerebro-spinal fluid	[100 - 300]
Amnionflüssigkeit/ amniotic fluid	[200 - 10000]
Samenflüssigkeit/ seminal plasma	[5000 - 15000]
Muttermilch/ breast milk	[1500-3000]
Zellkulturüberstand/ cell culture supernatants	[5 - 300]

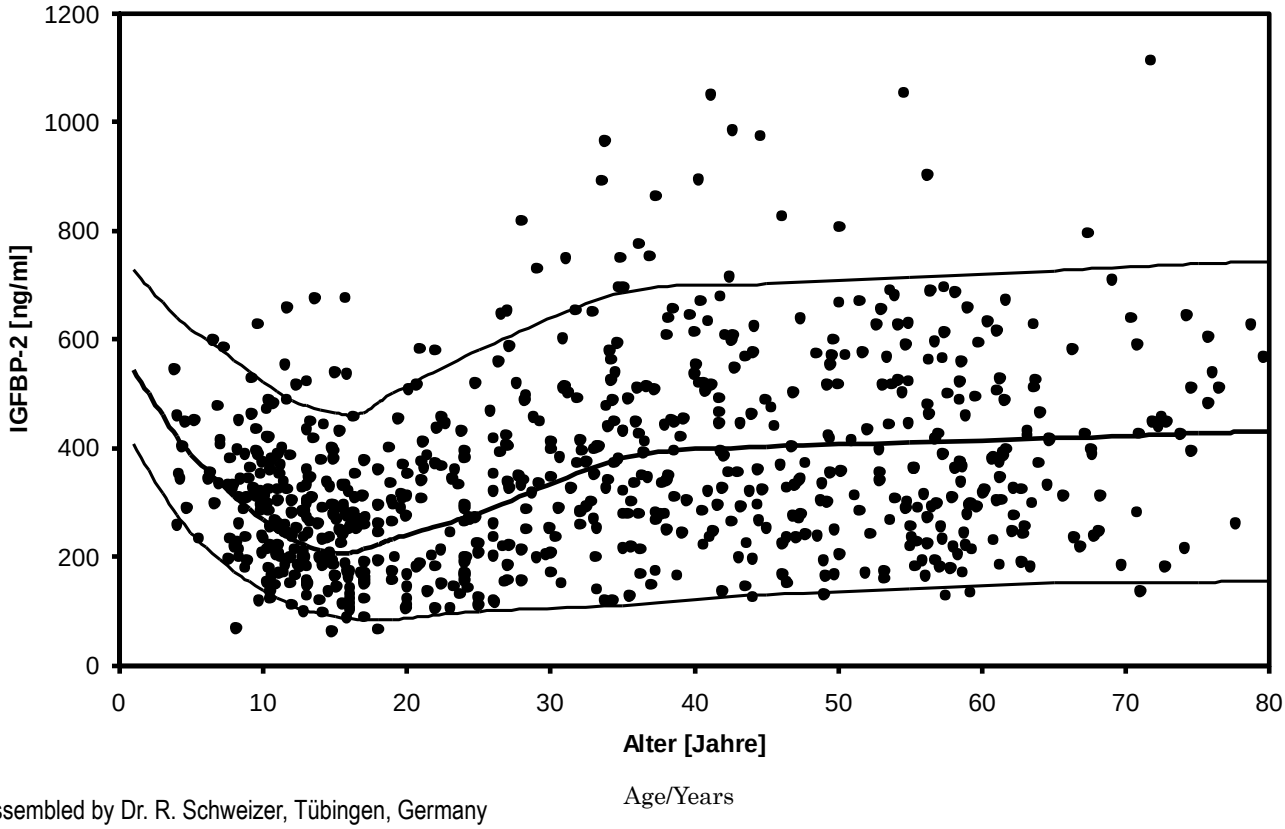
Table 7: Exemplary results of IGFBP-2 measurements in human adults between 20 and 80 years in dependence of the body mass index.

BMI		**Mittelwert mean**	SA	**Perzentilen percentiles**		
[kg/m²]	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 distribution of IGFBP-2 concentration is shown by the 5., 50. and 95. percentile for age classes.

Age-dependent distribution of IGFBP-2 in human serum samples.

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

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

REFERENCE

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2. Hoeflich A, Nedbal S, Blum WF, Erhard M, Lahm H, Brem G, Kolb HJ, Wanke R, Wolf E. (2001) Growth inhibition in giant growth hormone transgenic mice by overexpression of insulin-like growth factor-binding protein-2. Endocrinology 142:1889-98
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SUMMARY –IGFBP-2 ELISA

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.		

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 ↔ 50 rpm

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

0.5 h °C 20-25 ↔ 50 rpm

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

15 min °C 20-25

100 µl	H_2SO_4 SL
	MEASURE

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