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

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 (100 and 200µl) 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)
Plate washer and plate shaker (recommended)
Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)
Foil welding device for laminate bags (recommended)

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic 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 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. 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 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).
For clinical purposes we recommend a standard dilution of **1:21.**
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 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

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 month 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!

Substrat

The Substrat Solution S, stabilised H_2O_2- Tetramethylbenzidine, is photosenstive– Storage and Incubation in the dark.

Microtiterplate

Unused microtiterplate 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 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 **100µl** 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 **3 times** with **250 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 3 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 exceed 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** (MA) value for the blank from the duplicated determination (well A1/A2).

2) Subtract the mean absorbance (MA) 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 on semi-log paper (lin-log).

4) Recommendation: Calculation of the standard curve should be done by using a computer program. **Non-linear regression**, a higher-grade polynomial or four parametric logistic (4-PL) curve fit are in general suited for the evaluation.

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

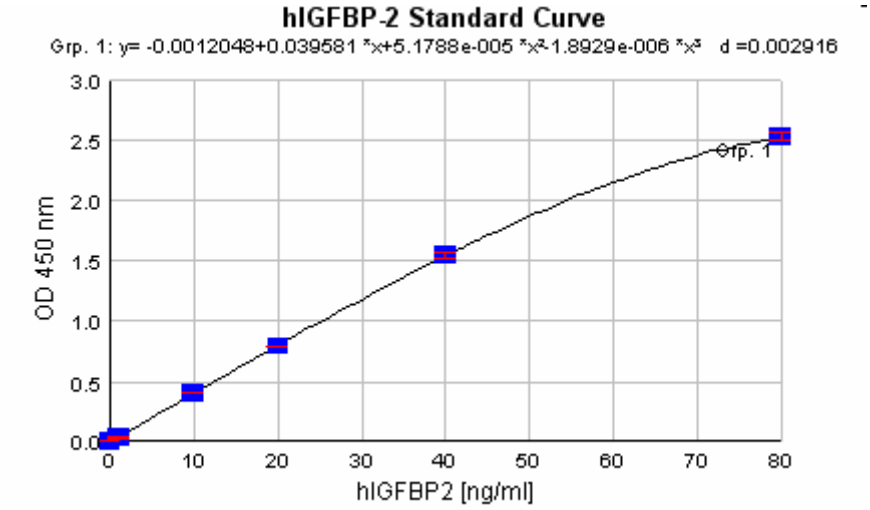


Fig. 1: Exemplary Standard Curve with a polynomial 3rd degree as curve fit.

PERFORMANCE CHARACTERISTICS

Standards

The Standards of the ELISA 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 is no cross-reactivity with IGFBP-1 nor 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 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

NIBSC 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:

Probenmaterial 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]

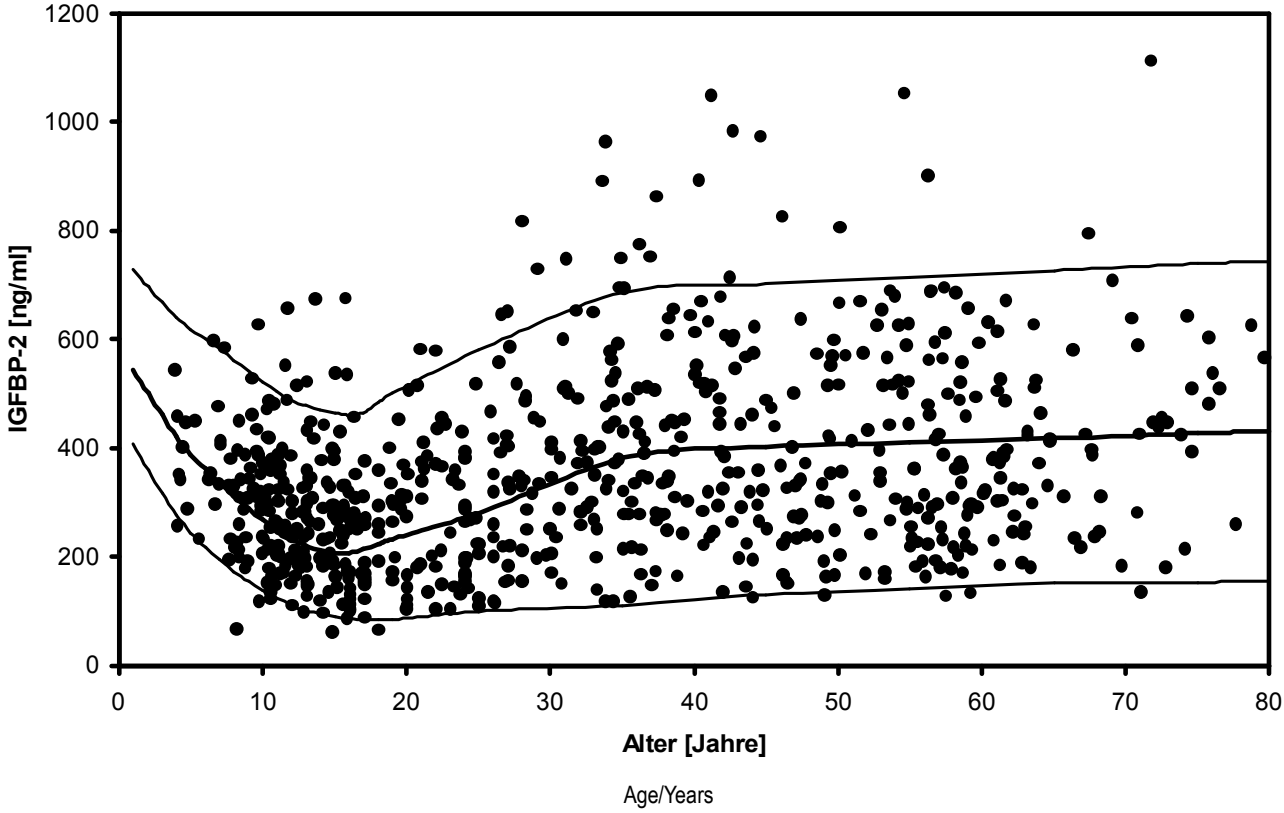
Table 7: BMI dependent reference values, adults between 20 and 80 years

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



Assembled by Dr. R. Schweizer, Tübingen, Germany

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.

REFERENCE

1. *Elmlinger MW, Wimmer K, Biemer E, Blum WF, Ranke MB, Dannecker GE, (1996) Insulin-like growth factor binding protein 2 is differentially expressed in leukaemic T-and B-cell lines. Growth Regulation 6, 152-157*
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*
3. *Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, Wollmann HA (2000) Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. Hormone Research 54: 60-68*
4. *Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, Wollmann HA (2001). Relevance of IGF-I, IGFBP-3, and IGFBP-2 measurements during GH treatment of GH-deficient and non-GH-deficient children and adolescents. Hormone Research 55: 115-124*
5. *Crofton PM, Ahmed SF, Wade JC, Elmlinger MW, Ranke MB, Kelnar CJH, Wallace WHB (1999) Effects of a third intensification block of chemotherapy on bone and collagen turnover, insulin-like growth factor I, its binding proteins and short term growth in children with acute lymphoblastic leukemia. European Journal of Cancer 35: 960-967*
6. *Muller HL, Oh Y, Lehrnbecher T, Blum WF, Rosenfeld RG.(1994) Insulin-like growth factor-binding protein-2 concentrations in cerebrospinal fluid and serum of children with malignant solid tumors or acute leukemia. J Clin Endocrinol Metab. 79: 428-34*
7. *Martin W Elmlinger, Martin H Deininger, Burkhardt S Schuett, Richard Meyermann, Frank Duffner, Ernst H Grote, and Michael B Ranke (2001) In vivo expression of the insulin-like growth factor binding protein-2 in human gliomas increases with the tumor grade. Endocrinology 142: 1652-1658*
8. *Cohen P, Peehl DM, Stamey TA, Wilson KF, Clemmons DR, Rosenfeld RG (1993) Elevated levels of insulin-like growth factor-binding protein-2 in the serum of prostate cancer patients. J Clin Endocrinol Metab. 76: 1031-1035*
9. *Boulle N, Baudin E, Gicquel C, Logie A, Bertherat J, Penfornis A, Bertagna X, Luton JP, Schlumberger M, Le Bouc Y. (2001) Evaluation of plasma insulin-like growth factor binding protein-2 as a marker for adrenocortical tumors. Eur J Endocrinol. 144: 29-36*
10. *Ranke MB, Maier KP, Schweizer R, Stadler B, Schleicher s, Elmlinger MW, Flehmig B (2003), Pilot study of elevated levels of insulin-like growth factor-binding protein-2 as indicators of hepatocellular carcinoma. Horm Res 60:174-180*
11. *Rosenfeld RG, Roberts CT Jr. (eds.) (1999) The IGF system: Contemporary Endocrinology Series; Humana Press*
12. *Jones JI, Clemmons DR. (1995) Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev. 16: 3-34*
13. *Chard T (1994) Insulin-like growth factors and their binding proteins in normal and abnormal fetal growth. Growth Reg 4: 91-100*
14. *Ranke MB, Elmlinger MW (1997) Functional role of insulin-like growth factor binding proteins. Hormone Research 48 (suppl 4): 9-15*
15. *Elmlinger MW, Bell M, Schütt, BS, Langkamp M, Kutoh E, Ranke MB (2001) Transactivation of the IGFBP-2 promoter in human tumor cell lines. Molecular and Cellular Endocrinology 175: 211-218*
16. *Hoeflich A, Reisinger R, Lahm H, Kiess W, Blum WF, Kolb HJ, Weber MM, Wolf E. (2001) Insulin-like growth factor-binding protein 2 in tumorigenesis: protector or promoter? Cancer Res.15: 8601-8610*
17. *Miell JP, Langford KS, Jones JS, Noble P, Westwood M, White A, Nicolaides KH. (1997) The maternal insulin-like growth factor (IGF) and IGF-binding protein response to trisomic pregnancy during the first trimester: a possible diagnostic tool for trisomy 18 pregnancies. J Clin Endocrinol Metab.82: 287-292*
18. *Elmlinger MW., Regine Grund, Michael Buck, Hartmut A Wollmann, Norman Feist, Matthias M. Weber, Christian C. Speer and Michael B. Ranke (1999). Limited proteolysis of the insulin-like growth factor binding protein (IGFBP-)2 by a specific serine protease activity in early breast milk. Pediatric Research 46: 76-81*
19. *Elmlinger MW, Michael S Sanatani, Mathias Bell, Günther E Dannecker, Michael B Ranke (1998) Elevated insulin-like growth factor (IGF) binding protein (IGFBP)-2 and IGFBP-4 expression of leukemic T-cells is affected by autocrine/paracrine IGF-II action but not by IGF type I receptor expression. European Journal of Endocrinology 138: 337-343*
20. *van den Beld, Blum W, Pols H, Grobbee D, Lamberts S (2003) Serum insulin-like growth factor binding protein-2 levels as an indicator of functional ability in elderly men.European Journal of Endocrinology 148: 627-634*

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 upm		
3x 250 µl	Aspirate the contents of the wells and wash **3x** with **250 µl Wash Buffer WP**	each well
100 µl	**Antibody-POD-Conjugate AK**	each well
Incubation: 30 min at RT, ≥ 350 rpm		
3x 250 µl	Aspirate the contents of the wells and wash **3x** with **250 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.		

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

3x 250 µl	3x WASHBUF WP
100 µl	AbCONJ AK
	TAPE

0.5 h °C 20-25 ↔ ≥ 350 rpm

3x 250 µl	3x WASHBUF WP
100 µl	SUBST TMB S

15 min °C 20-25

100 µl	H_2SO_4 SL
	MEASURE

Distributed by

BioVendor - Laboratorní medicína a.s.
CTPark Modrice
Evropska 873
664 42 Modrice
CZECH REPUBLIC
Phone: +420-549 124 185
Fax: +420-549 211 460
e-mail: info@biovendor.com
http://www.biovendor.com

European Union:

BioVendor GmbH
Im Neuenheimer Feld 583
D-69120 Heidelberg, GERMANY
Phone: +49-6221-433-9100
Fax: +49-6221-433-9111
e mail: roth@biovendor.com
e mail: abels@biovendor.com

China - Hong Kong Office:

BioVendor Laboratories Ltd.
Room 4008, Hong Kong Plaza, No.188
Connaught Road West
Hong Kong, CHINA
Phone: +852 28030523
Fax: +852 28030525
e-mail: lu@biovendor.com

USA, Canada and Mexico:

BioVendor, LLC
1463 Sand Hill Road, Suite 227
Candler, NC 28715, USA
Phone: +1-828-670-7807, +1-800-404-7807
Fax: +1-828-670-7809
e mail: info-us@biovendor.com

China – Mainland Office:

BioVendor Laboratories Ltd.
Room 2405, YiYa Tower, TianYu Garden, No.150
Lihe Zhong Road, Guang Zhou, CHINA
Phone: +86-20-87063029
Fax: +86-20-87063016
e-mail: jhsu@biovendor.com

Manufactured by

Aspenhausttr. 25
D-71770 Reutlingen
Germany
http://www.mediagnost.de
e-mail: contact@mediagnost.de