IGFBP-3 ELISA

Enzyme-Immunoassay for Quantitative Determination of

Insulin-Like Growth-Factor Binding Protein-3

Product-Code: E03

CE

DE/CA40/00809/22

For In-Vitro Use Only !

In the USA: For Research Use Only

mediagnost

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FEATURES

- ♦ Quantitative determination of IGFBP-3 without sample pretreatment
- ♦ Inter-Assay variation of 6.30% and Intra-Assay variation of 4.51%
- ♦ Sensitivity of 0.6 ng/ml
- ♦ Measures growth hormone (GH)-dependent IGFBP-3 Bindingprotein
- ♦ Stable serum levels due to absence of circadian variation
- ♦ Integrates the GH secretory state over days
- ♦ A single measurement is highly informative for diagnosis of GH deficiency or GH excess
- ♦ Ideal for the diagnosis of GH-deficiency in young children
- ♦ Small sample requirement, thus ideal for pediatric patients.

INTRODUCTION

Insulin-like growth factors (IGF)-I and -II are bound to specific binding proteins (IGFBPs) in the circulation. To date, at least six binding proteins can be distinguished on the basis of their amino acid sequence. They are designated as IGFBP-1, IGFBP-2, ... IGPBP-6 (1). Lately the discovery of a new IGFBP-7 has been discussed (2). The predominating IGFBP in blood is IGFBP-3, which largely determines the total IGF-I and IGF-II concentration. In contrast to the other binding proteins, IGFBP-3 has the unique property to associate with an acid-labile non-binding subunit (ALS) after binding of either IGF-I or IGF-II (3-5). Most of the IGFBP-3 in plasma is present as the high molecular weight ternary complex, however, small amounts of free IGFBP-3 are also found (6,7).

The development of specific immunoassays for IGFBP-3, those also recognize the complete high molecular weight complex, provided new in-sights into its regulation (6-9). On the basis of these findings se-

rum IGFBP-3 has proved to be an additional useful test in the repertoire of diagnostic tools for evaluation of growth disorders (7,8).

Several factors besides GH influence IGFBP-3 levels: age including sexual development, nutrition, hypothyroidism, diabetes mellitus, liver function and kidney function. IGFBP-3 levels are decreased by malnutrition, although less than IGF-I, in hypothyroidism, in diabetes mellitus and in hepatic failure (6-8), but are increased in chronic renal failure (6,10,11). Measurement over 24 hours revealed constant circadian levels (12,13). For clinical practice, the most important regulatory factor is GH. Single IGFBP-3 measurements correlate significantly with the logarithm of the integrated spontaneous GH secretion (8,14). In patients with GH deficiency, IGFBP-3 levels are subnormal and increase gradually to within the normal range after several days of GH administration (7,8). The slow response to GH and constant circadian levels during chronic daily application of GH (13) suggest that IGFBP-3 reflects the GH secretory state over days.

So far, IGF-I serum levels have been widely used in screening for GH deficiency or acromegaly. However, several limitations are obvious:

1. The normal range of IGF-I is low in young children making discrimination of subnormal levels difficult at that age.
2. A considerable number of children of small stature have, despite normal GH secretion, IGF-I levels in the subnormal range. Therefore, the specificity and consequently the accuracy of the test for diagnosis of GH deficiency are limited.

The major advantages of IGFBP-3 over IGF-I are:

1. No extraction step is required prior to measurement (as is still necessary in certain IGF-I assays) thus improving test accuracy by simplifying the assay procedure.
2. The normal range in young children is comparatively high making the detection of subnormal levels more reliable.
3. Patients with GH deficiency have subnormal IGFBP-3 levels. In contrast, most of the small statured children with normal GH secretion have levels within the normal range (Figure 1). The separation of these two groups is easy. A single measurement of the IGFBP-3 concentration is sufficient for the diagnosis of GH deficiency with high accuracy (7,18). In small statured children IGFBP-3 levels rise to normal range within several days of GH administration and remain normal during continuous GH treatment (Figure 2). Therefore, serum IGFBP-3 measurements are also suited for evaluating the potential of a patient to respond to GH and for GH therapy monitoring (19). In other patients of severe short stature, e.g. Ullrich-Turner syndrome or Silver-Russell syndrome, IGFBP-3 levels were found normal (8) reflecting normal GH secretion.

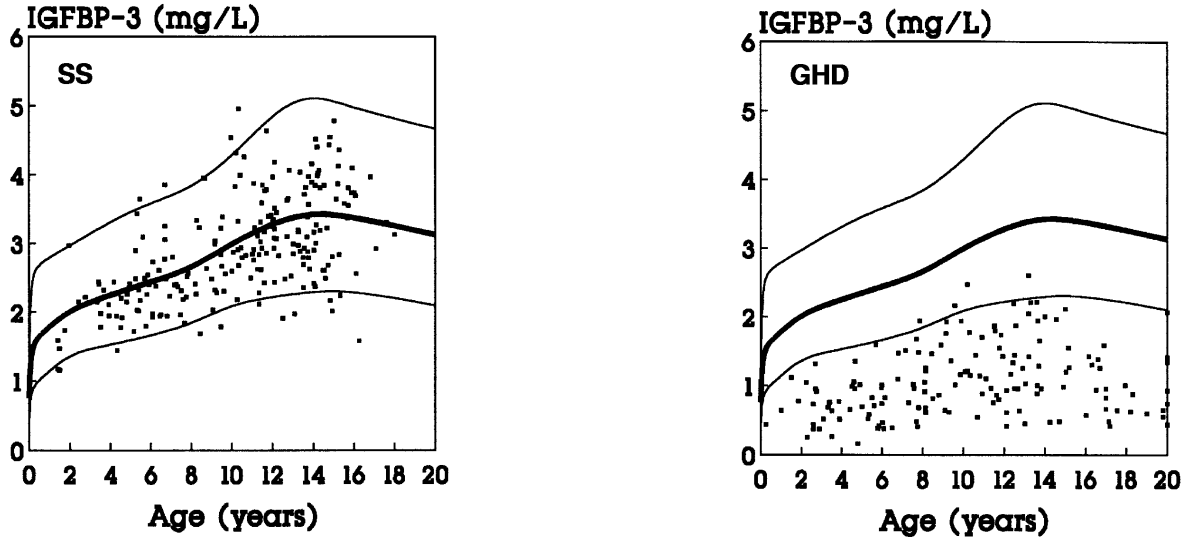

Figure 1: Serum IGFBP-3 levels in patients with short stature without GH deficiency (SS: constitutional delay of growth and adolescence, familial short stature, intrauterine growth retardation) and in idiopathic or organic GH deficiency (GHD). The normal range is given by the 5th, 50th and 95th percentile.

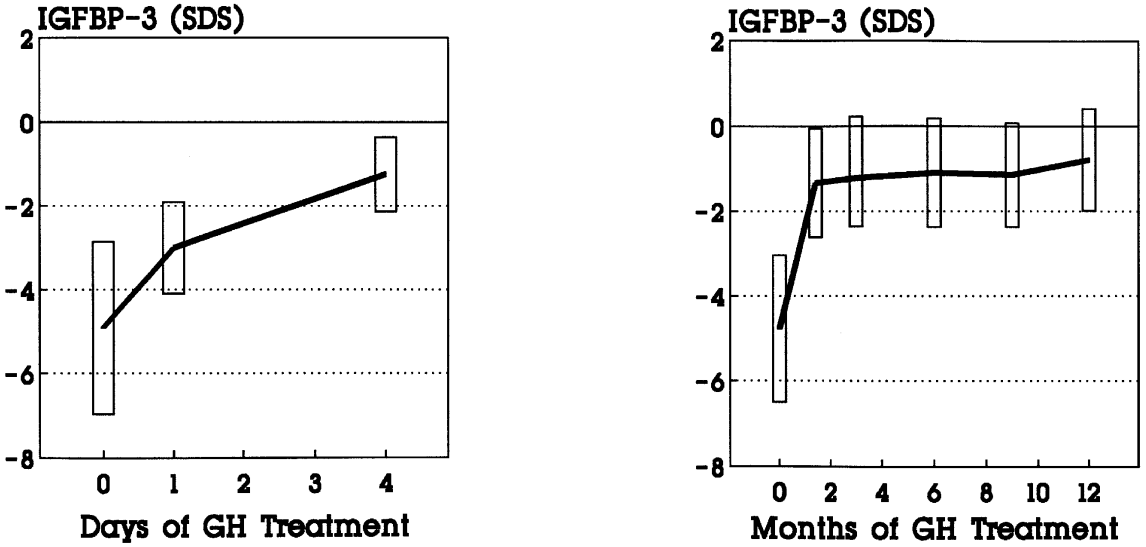

Figure 2: IGFBP-3 levels in GH deficient children before and during GH treatment. Because of the age-dependence, values are given as the mean of standard deviation scores (SDS).

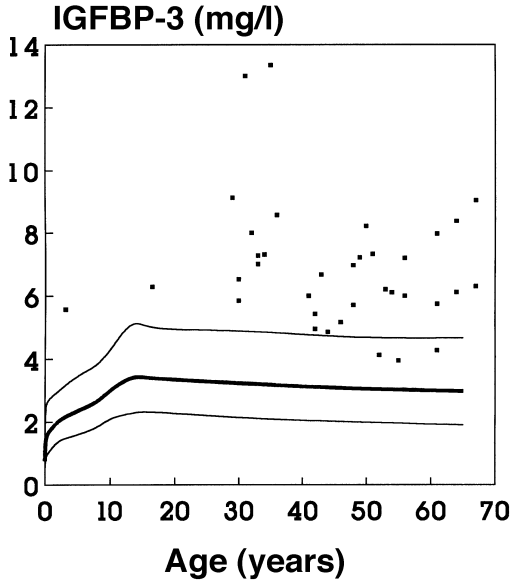

Figure 3: Serum IGFBP-3 levels in acromegaly. The normal range is given by the 5th, 50th and 95th percentile.

In normal tall children and adolescents without excessive GH secretion or in patients with Sotos syndrome, IGFBP-3 levels are normal or slightly increased. In contrast, children with pituitary gigantism or adults with acromegaly have clearly elevated levels (Figure 3) (6,15) that normalize on successful treatment. Therefore, IGFBP-3 is also a useful parameter for the detection of excessive GH secretion and monitoring therapy efficacy. In precocious puberty, IGFBP-3 levels are clearly increased by chronological age, whereas patients with premature thelarche have IGFBP-3 levels in the upper normal range (15).

INTENDED USE

This enzyme immunoassay kit is suited for measuring IGFBP-3 in human serum or Heparin-plasma for diagnostic and scientific purposes.

Its diagnostic value for GH deficiency screening is based on the high sensitivity and specificity of serum IGFBP-3 as a test for this diagnosis. States of GH excess may also be detected since IGFBP-3 levels

are increased in that case. Due to its constant circadian concentration IGFBP-3 determination in a single blood sample may be sufficient as a screening test for these pathological situations prior to subjecting patients to further testing of GH secretion. IGFBP-3 determinations may also be suited for monitoring the efficacy of treatment and the patient's compliance in GH deficiency and acromegaly.

Assay Characteristics and Validation

The Mediagnost ELISA for IGFBP-3 E03 is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGFBP3 in the sample binds to the immobilized first antibody on the microtiter plate. In the following step, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-IGFBP-3-Antibody binds in turn to the immobilised IGFBP-3. In the closing substrate reaction the turn of the colour will be high specific catalysed, quantitatively depending on the IGFBP-3-level of the samples.

The standards of the ELISA E03 are **native human IGFBP-3** in concentrations of **2, 10, 30, 75 and 150 ng/ml**.

The **analytical sensitivity** of the ELISA E03 yields **0.6 ng/ml** (2 SD of zero standard in 18fold determination).

The Mediagnost IGFBP-3 ELISA E03 is over a very wide range dilution true. The **Linearity of the dilution of the sera** is excellent (s. Table 1).

Table 1: The linearity of the sample dilution

(representative results of two different sera are listed)

Dilution:	Sample 1 (recalculated, ng/ml)	Dilution:	Sample 2 (recalculated, ng/ml)
1:20	3250	1:20	3078
1:40	3489	1:40	3179
1:80	3181	1:80	3221
1:160	3167	1:160	3402
1:320	3013	1:320	3066
1 :640	2936	1 :640	2901
1 :1280	2895	1 :1280	3364
AV / 1SD / VC%	3133 / 205 / 6,54	**AV / 1SD / VC%**	3173 / 176 / 5.55

AV = Average Value, **SD** = Standard deviation **VC** = Variation Coefficient%

The **Inter**- and **Intra-Assay** variation coefficients were found less than **6.30% and 4.51%**. Exemplary determinations are shown in table 2 and table 3.

Table 2 : Inter-Assay-Variation (n=9)

	Mean Value (ng/ml)	Standard Deviation (ng/ml)	VC (%)
Sample 1	**2568**	**148**	**5.76**
Sample 2	**3334**	**210**	**6.30**
Sample 3	**4082**	**233**	**5.70**

Table 3: Intra-Assay-Variation (n=16)

	Mean Value (ng/ml)	Standard Deviation (ng/ml)	VC (%)
Sample 1	**1764**	**76.6**	**4.34**
Sample 2	**2260**	**98.5**	**3.96**
Sample 3	**3699**	**167.0**	**4.51**

Clinical Validation

Clinical validation was achieved by determination of IGFBP-3 levels in a large number of normal children and adults, normal short statured children without GH deficiency, girls with Ullrich-Turner Syndrome, children with Silver-Russell Syndrome, patients with GH deficiency, children with familial tall stature, Sotos-Syndrome, patients with acromegaly, children with premature thelarche and precocious puberty (Tab. 4; Abb. 1, 2, 3, 4 und 5).

Sampels: Applicability, Preparation and Storage

Serum samples and Heparin-Plasma samples are suitable. A special external sample preparation prior to assay is not required. Results in Citrat- or EDTA-Plasma are about 15% reduced. Slight Hemolysis of the samples doesn´t disturb the determination.

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen -20°C or below in tightly closable plastic tubes. Avoid on principal 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-3 determinations in small sample volumes, which is limited by pipetting accuracy rather than the amount of IGFBP-3.

In most determinations (e.g. Serum- or Plasma samples and no extreme values expected) the dilution of **1:101 with Sample Buffer PP is suitable**, the respective covered range would be 0.2 to 15.15 mg/L. Where required, depending on the expected IGFBP-3-values, the dilution with **Sample Buffer PP** can be higher or lower. The IGFBP-3 concentrations maybe completely different in body fluids of human origin other than serum or in cell culture supernatants.

Suggestion for dilution protocol:
Pipette **1 ml Sample Buffer PP** (green colored) in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µl Serum**- or **Plasma** (dilution 1:101) and mix each tube **immediately**. After mixing use **10 µl** of this solution within 1 hour **per determination** in the assay (pipetting control = blue coloring of the solution in the wells).

MATERIALS

Materials Provided

1) **Microtiter plate**, ready for use, Microtiter plate with **96 wells**, divided up in 12 strips à 8 wells (**separately breakable**). Coated with an antibody against human IGFBP-3.
2) **Standards A-E**, lyophilised, contain native human IGFBP-3. Standard values are between **2 - 150 ng/ml** (2, 10, 30, 30, 75 and 150 ng/ml) IGFBP-3, Standards are reconstituted with **250 µl Sample Buffer PP** each. Use **10 µl pro** well in the assay.
3) **Antibody Conjugate AK,** 140 µl, **100-fold Concentrate,** contains biotinylated anti-human IGFBP-3 Antibody.
4) **Enzyme Conjugate EK,** 140 µl, **100-fold Concentrate**, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin.
5) Please mix the **Antibody Conjugate AK** and the **Enzyme Conjugate EK** before use **together 1:100** in **Dilution Buffer VP**, for this pipette at first AK in VP and mix, add then EK (e.g.

100 µl **AK** + 9,8 ml **VP,** + 100 µl **EK**). Pipette **100 µl** of the **AK/EK-solution** per well.

6) **Dilution Buffer VP**, 30 ml, ready for use, please use for dilution of the **Antibody Conjugate AK** and the **Enzyme Conjugates EK** .
7) **Sample Buffer PP**, 120 ml, ready for use, green colored, please use for the **dilution** of **Samples, Standards** and **Control**.
8) **Control Serum KS**, 250 µl**,** lyophilised, contains human Serum and should be reconstituted in **250 µl Sample Buffer PP**. The IGFBP-3 target value and the respective range are given on the vial label. The dilution should be according to the dilution of the respected samples.
9) **Washing Buffer**, 50 ml, 20-fold concentrated solution, please dilute **before use 1:20 with A.dest**. or demineralised water (e.g. add the complete contents of the flask, 50 ml, into a graduated flask and fill with A.dest. to 1000 ml). Attention: after dilution, the Washing Buffer is only limited stable; please dilute only according to requirements.
10) **Substrate S,** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2-Tetramethylbencidine.
11) **Stopping Solution SL**,12 ml, ready for use, 0.2 M sulphuric acid, *Caution*!
12) **Sealing tape** for covering of the microtiter plate, 2 x, adhesive.

Technical Recommendations

In conducting the assay, follow strictly the test protocol.

Reagents with different lot numbers should not be mixed. The microtiter plate and all reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

The Standards **A – E** and **Control Serum KS** are reconstituted with the **Sample Buffer PP** provided in the Kit. It is recommended to keep the reconstituted reagents at room temperature for 15 minutes

and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer. Use the **Dilution Buffer VP** for the dilution of **Antibody** and **Enzyme Conjugate concentrates (AK and EK).**

The shelf life of the components after opening is not affected, if used appropriately. Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C. Reconstituted Components (Standards A – E and Control Serum KS) should be stored at 2-8°C for up to 1 week. If longer storage time is needed, store the components at -20°C (or below). Freezing extends the expiry at least 2 months. Avoid repeated freeze-thaw cycles. In case you plan to perform multiple independent IGFBP-3-determinations over a longer period with one kit, you should aliquot the components prior to freezing into suitable smaller volumes.

The 1:20 diluted **Washing Buffer WP** is only limited stable, please dilute only according to requirements. This applies to the 1:100 diluted mix of **Antibody Conjugate AK** and **Enzyme Conjugate EK** too.

Before use, all kit components should be brought to room temperature. **Precipitates, possible in buffers, should be dissolved before use through mixing and warming**.

Room temperature incubation means: Incubation at 20 - 25°C.

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

When performing the assay, the Standards **(A-E),** Control Serum **(KS)** and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times the mix of Antibody Conjugate **AK** and Enzyme Conjugate **EK** as well as the succeeding **Substrate Solution S** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution SL** should be added to the plate in the same order as the Substrate Solution **S**.

Materials not Provided

Distilled or demineralised water for dilution of the Washing Buffer WP

Micropipettes and multichannel pipettes with disposable plastic tips
Vortex-mixer
Device to aspirate the samples from the wells (recommended because of the potential danger of infection by human samples)
Plate washer and plate shaker (recommended)
Microplate reader ("ELISA-Reader") with Filter for 450 and ≥590 nm.
Foil welding device for laminate bags (recommended)

PRECAUTIONS AND WARNINGS

The **mediagnost IGFBP-3 ELISA, E03** is for in-vitro use only! This product has to be used as described in the enclosed instructions. The Mediagnost GmbH is not liable for any loss or harm caused by non-observance of the instructions, as far as no law withstands.

Caution: This kit contains material of human and/or animal origen. All components have to be treated as potentially infectious.

Reasonable precautions have to be taken and rules of good laboratory practice are to apply regarding storage, use and waste disposal of all kit components. Waste disposal has to be done in accordance with local regulations.

Human Serum:

Contained in following components: **Standards A-E**, **KS**

Human material, used for preparation of this products was tested by regulatory accredited methods for antibodies against human immunodeficient virus (HIV I and II), against hepatitis B virus surface antigen and against hepatitis C virus and shown as negative for all tested antibodies. No test method exclude the presence of infectious pathogens totally, therefore all reagent should be treated according the guidelines of biological safety level 2.

Kathon CG

Contained in: **AK, EK, VP, WP, PP**

< 0.1% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one und 2-methyl-2H-isothiazol-3-one

Germall II
Contained in: **AK, EK, VP, PP**
< 0.1% Diazolidinyl Urea

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

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
S36/37	Wear suitable protective clothing and gloves

Stop Solution (SL)
Stop solution contains 0,2 M Sulfur 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
S36/37	Wear suitable protective clothing and gloves.

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. The Stop Solution pro-

vided is an acid solution. Avoid direct contact. Wear eye, hand, face and clothing protection when using this material.

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ASSAY PROCEDURE

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

1) Please pipette on before in **all needed wells 100 µl Dilution Buffer VP**.
2) Add **10µl Sample Buffer PP** in positions A1/2
3) Pipette in positions B1/2 **10µl** each **Standard A (2 ng/ml)**,
   pipette in positions C1/2 **10µl** each **Standard B (10 ng/ml)**,
   pipette in positions D1/2 **10µl** each **Standard C (30 ng/ml)**,
   pipette in positions E1/2 **10µl** each **Standard D (75 ng/ml)**,
   pipette in positions F1/2 **10µl** each **Standard E (150 ng/ml)**,
   To control the correct accomplishment **10 µl** of the 1:101 (or in respective dilution rate of the sample) in Sample Buffer **PP** diluted **Control Serum KS** can be pipetted in positions G1/2.
   Pipette **10 µl each** of the **diluted sample** (generally 1:101 diluted in Sample Buffer **PP**) in the rest of the wells, according to requirements. Please mix the dilutions immediately after sample addition and use within 60 minutes.
4) Cover the wells with the sealing tape and incubate the plate for **1 hour** at **room temperature** (if possible, shake at ≥350 rpm).
5) After incubation aspirate the contents of the wells and wash the wells 3 times with **250 µl Washing Buffer WP**.
6) Following the last washing step, pipette **100 µl** of the **1:100 jointly diluted Antibody Conjugate AK and Enzyme Conjugate EK Mix** in each well.
7) Cover the wells with the sealing tape and incubate **1 hour** at **room temperature** (if possible shake at ≥350 rpm).

8) After incubation wash the wells 3 times with **Washing Buffer WP** as described in step 5)
9) Pipette **100 µl of the TMB-Substrate solution S** in each well.
10) Incubate the plate for **30 Minutes in the dark at** room **temperature**.
11) After incubation pipette **100 µl Stop Solution SL** in each well.
12) Measure the absorbance **within 30 minutes at 450 nm (Reference filter ≥590 nm**, **e.g. 620 nm)**.

EVALUATION

Establishing the Standard Curve

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be 0.2, 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.

The standards provided contain the following concentrations of IGFBP-3:

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	2	10	30	75	150

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.

4) Recommendation: Calculation of standard curve should be done by using a computer programme, because the curve is in general (without respective transformation) not ideally described by linear regression. A higher-grade polynomial or four parameter logistic (4PL) lin-log curve fit are suitable for the evaluation.

5) The IGFBP-3 concentration in ng/ml of the samples can be calculated by multiplication with the respective dilution factor, Division by 1000 converts the values in µg/ml or equal mg/Litre (Example: a measured value was 30 ng/ml, Sample was 1:101 diluted: 30 x 101= 3030 ng/ml, or 3,03 µg/ml or 3,03 mg/L, according the requested unit).

EXPECTED VALUES

IGFBP-3-levels are strongly age-dependent in children, less so in adults. The normal ranges in various age-groups which were log-normally distributed are given in table 4 by the percentiles. A graphic presentation is shown in Fig.4 and 5. It is recommended for each laboratory to establish its own normal range.

LIMITATIONS

IGFBP-3 levels are strongly dependent on GH secretion. However, a number of factors influence its plasma concentration and should be taken into account for appropriate interpretation. Plasma levels decrease during fasting (more than 1 day), in malnutrition, malabsorption, cachexia, impaired hepatic function, hypothyroidism, and diabetes mellitus. They may also be decreased in chronic inflammatory disease and malignancy. Levels are increased in states of impaired renal function and precocious puberty. In clinical situations with hyperprolactinemia or in patients with craniopharyngeoma, normal levels may be observed despite GH deficiency.

In certain physiological (e.g. pregnancy) and pathological states, IGFBP-3 may be degraded to smaller molecular size compounds (16,17) by specific proteases which affect IGFBP patterns seen in Western ligand blotting, but in general only have little influence on the outcome of ELISA determinations. In case of special interest in this physiological process, the Mediagnost ELISA for **functional IGFBP-3 E04** is available. The ELISA E04 enables to quantify the degree of **IGFBP-3 fragmentation** in samples.

Tab. 4: Serum levels of IGFBP-3 in healthy subjects at various ages. Individuals between 7 and 17 years of age were classified according to gender, as the pubertal peak occurs almost 2 years earlier in girls than in boys.

	Percentile													
Age group	**0.1**	1	**5**	10	**20**	30	**40**	50	**60**	70	**80**	90	**95**	99
0-1 week	**0.25**	0.33	**0.42**	0.48	**0.57**	0.64	**0.70**	0.77	**0.85**	0.93	**1.05**	1.23	**1.41**	1.81
1-4 weeks	**0.49**	0.62	**0.77**	0.86	**0.99**	1.10	**1.19**	1.29	**1.40**	1.52	**1.68**	1.93	**2.16**	2.68
1-3 months	**0.55**	0.70	**0.87**	0.98	**1.13**	1.25	**1.36**	1.48	**1.61**	1.75	**1.94**	2.23	**2.52**	3.14
3-6 months	**0.64**	0.80	**0.98**	1.10	**1.25**	1.38	**1.49**	1.61	**1.74**	1.88	**2.07**	2.37	**2.65**	3.24
6-12 months	**0.71**	0.88	**1.07**	1.19	**1.35**	1.48	**1.60**	1.72	**1.85**	2.00	**2.19**	2.49	**2.76**	3.36
1-3 years	**1.02**	1.21	**1.41**	1.53	**1.69**	1.82	**1.94**	2.05	**2.17**	2.31	**2.48**	2.74	**2.98**	3.47
3-5 years	**1.08**	1.30	**1.52**	1.66	**1.84**	1.99	**2.12**	2.25	**2.39**	2.55	**2.75**	3.05	**3.33**	3.91
5-7 years	**1.19**	1.42	**1.66**	1.81	**2.01**	2.16	**2.30**	2.44	**2.59**	2.76	**2.97**	3.29	**3.59**	4.2

7-9 y.	boys	**1.25**	1.48	**1.73**	1.88	**2.07**	2.22	**2.36**	2.50	**2.65**	2.81	**3.02**	3.33	**3.61**	4.22
	girls	**1.36**	1.61	**1.88**	2.04	**2.25**	2.42	**2.57**	2.72	**2.88**	3.06	**3.28**	3.62	**3.94**	4.58
9-11 y.	boys	**1.47**	1.73	**1.99**	2.15	**2.36**	2.52	**2.66**	2.81	**2.96**	3.14	**3.35**	3.67	**3.97**	4.57
	girls	**1.56**	1.90	**2.20**	2.38	**2.62**	2.80	**2.96**	3.13	**3.30**	3.50	**3.75**	4.11	**4.45**	5.16
11-13	boys	**1.58**	1.88	**2.19**	2.38	**2.63**	2.82	**3.00**	3.18	**3.37**	3.58	**3.84**	4.25	**4.62**	5.39
	girls	**1.62**	1.90	**2.24**	2.46	**2.74**	2.97	**3.17**	3.38	**3.60**	3.85	**4.17**	4.65	**5.10**	6.02
13-15 y.	boys	**1.62**	1.89	**2.24**	2.46	**2.76**	2.99	**3.20**	3.42	**3.65**	3.91	**4.24**	4.75	**5.22**	6.20
	girls	**1.69**	2.03	**2.39**	2.61	**2.91**	3.14	**3.35**	3.56	**3.79**	4.04	**4.36**	4.85	**5.30**	6.24
15-17 y.	boys	**1.70**	2.02	**2.36**	2.57	**2.84**	3.05	**3.25**	3.44	**3.65**	3.88	**4.17**	4.61	**5.01**	5.86
	girls	**1.62**	1.93	**2.26**	2.46	**2.73**	2.93	**3.12**	3.31	**3.51**	3.74	**4.02**	4.45	**4.85**	5.67
17-20 y.		**1.58**	1.90	**2.24**	2.45	**2.72**	2.94	**3.13**	3.33	**3.54**	3.78	**4.07**	4.53	**4.95**	5.83
20-30 y.		**1.55**	1.86	**2.20**	2.41	**2.68**	2.90	**3.09**	3.29	**3.50**	3.74	**4.04**	4.50	**4.92**	5.80
30-40 y.		**1.44**	1.75	**2.08**	2.29	**2.56**	2.78	**2.98**	3.18	**3.39**	3.64	**3.95**	4.42	**4.86**	5.78
40-50 y.		**1.38**	1.68	**2.01**	2.21	**2.48**	2.69	**2.88**	3.08	**3.29**	3.53	**3.83**	4.29	**4.72**	5.63
50-60 y.		**1.34**	1.64	**1.96**	2.16	**2.42**	2.63	**2.83**	3.02	**3.23**	3.46	**3.76**	4.22	**4.65**	5.55
60-70 y.		**1.28**	1.58	**1.90**	2.10	**2.37**	2.58	**2.78**	2.98	**3.19**	3.44	**3.75**	4.23	**4.67**	5.62

Serum levels are given as mg/L

Determined with IGFBP-3 RIA (Blum et al. 1990)

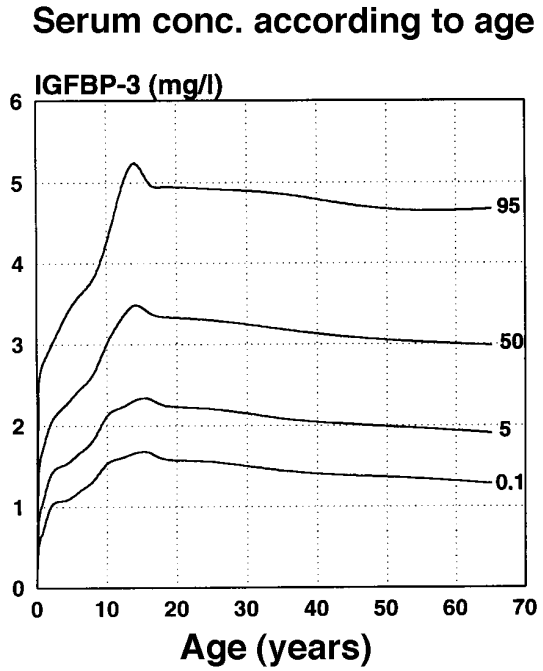

Fig. 4: : Age-dependant normal values of IGFBP-3 (presented as 0.1., 5., 50., and 95. percentile)

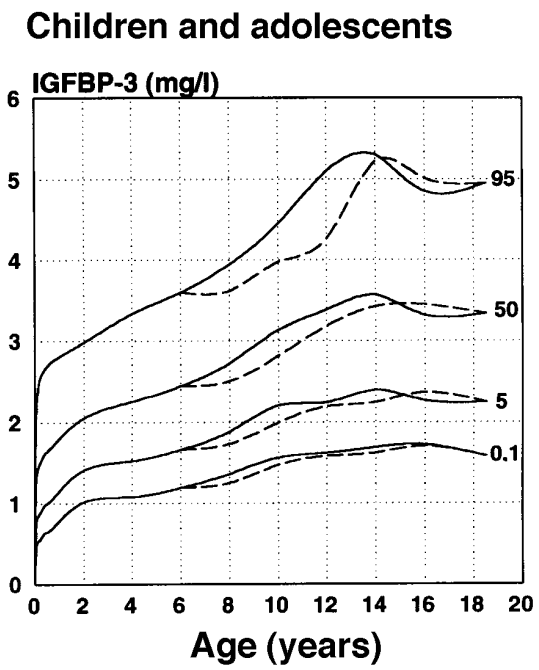

Fig. 5: Normal values of children and adolescents (girls ——— boys - - - -)

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SUMMARY – Mediagnost IGFBP-3-ELISA E03

Reconstitution/ Dilution of Reagents		
Standards A-E	Reconstitution in **Sample Buffer PP** (green)	**250 µl each**
Control Serum KS	Reconstitution in **Sample Buffer PP** (green)	**250 µl**
Antibody Conjugate AK + Enzyme Conjugate EK	Before use dilute AK and EK 1:100 each as Mix in **Dilution Buffer VP** – add at first AK in VP, mix, add then EK and mix again (e.g. 100 µl AK plus 9,8 ml VP, then add 100 µl EK)	**together 1:100 each**
Washing Buffer WP	dilute in **A. dest.** (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	**1:20**
Sample Dilution + Control Serum KS: 1:101 in Sample Buffer PP (green colored; e.g. 10 µl in 1 ml **PP**), **mix directly and use within max. 60 min.** Use **10 µl per determination** (pipetting control= blue coloration)		
Before assay procedure bring all **reagents** to **room temperature**		

Proposal of Assay Procedure for Double Determination:

Pipette	**Reagents**	**Well Positions**
100 µl	Dilution Buffer **VP**	Pipette **in all required number of wells**
10 µl	Sample Buffer **PP** as Blank	A1 and A2
10 µl	Standard **A** (**2 ng/ml**)	B1 and B2
10 µl	Standard **B** (**10 ng/ml**)	C1 and C2
10 µl	Standard **C** (**30 ng/ml**)	D1 and D2
10 µl	Standard **D** (**75 ng/ml**)	E1 and E2
10 µl	Standard **E** (**150 ng/ml**)	F1 and F2
10 µl	Control Serum **KS**	G1 and G2
10 µl	**Sample**	Pipette sample in the rest of the wells according to requirements
Cover the wells with the sealing tape		

Incubation: 1 h at RT. ≥ 350 rpm

3x 250 µl	Aspirate the contents of the wells and and wash **3x** with **250 µl** each **WP/well**	each well
100 µl	**Mix aus AK und EK**	each well

Incubation: 1 h at RT, ≥350 rpm

3x 250 µl	Aspirate the contents of the wells and wash **3x** with **250 µl** each **WP/well**	each well
100 µl	Substrate Solution **S**	each well

Incubation: 30 min in **the dark** at RT

100 µl	Stop Solution **SL**	each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)		