Human IGFBP – 3 total ELISA

(Human Insulin-like Growth Factor Binding Protein-3)

Cat. No.: RMEE03A

IVD CE

TECHNICAL FEATURES+APPLICATIONS

- For research use only!
- Quantitative determination of IGFBP-3 without sample pretreatment
- Inter-Assay variation of 6.30% and Intra-Assay variation of 4.51%
- Sensitivity of 0.1 ng/ml
- 2 Control Sera are provided for quality control purposes

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 to 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, recognizing the complete high molecular weight complex, provided new in-sights into ternary complex regulation (6-9).
Several factors besides GH influence IGFBP-3 levels: age including sexual development, nutrition, hypothyroidism, diabetes mellitus, liver function and kidney function.

Measurement over 24 hours revealed constant circadian levels (12,13).

INTENDED USE

This enzyme immunoassay kit is for research use and quantifies IGFBP-3 in human serum, Heparinplasma or in cerbrospinal fluid.

PERFORMANCE CHARACTERISTICS AND VALIDATION

The Mediagnost ELISA for IGFBP-3 E03A is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGFBP-3 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 E03A are of recombinant human **IGFBP-3** in concentrations of **0.4; 2; 6; 15 and 30 ng/ml**.

Sensitivity

The **analytical sensitivity** of the ELISA E03A yields **0.1 ng/ml** (2 SD of zero standard in 18fold determination).

Table 1: Linearity

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 = Coefficient of VariationTable

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

	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

	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

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum samples, Heparin-Plasma samples and Cerebrospinalfluid 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 **1:505 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** (yellow colored) in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µl Serum**- or **Plasma** (dilution 1:101). Add 400 µl Sample Buffer **PP** in an other PE-/PP-tube and 100 µl of the thoroughly mixed first dilution (dilution 1:5). After mixing use **50 µl** of this 1:505 diluted solution within 1 hour **per determination** in the assay (pipetting control = blue coloring of the solution in the wells).

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 anti-human IGFBP-3 Antibody, packed in a laminate bag.
2.	CAL	**Standards A-E**, lyophilised, contain human IGFBP-3. Standard values are between 0.4 - 30 ng/ml (0.4, 2, 6, 15 and 30 ng/ml) IGFBP-3, Standards are **reconstituted with 1 ml Sample Buffer PP each**. Use 50 µl pro well in the assay.
3.	BUF VP	**Dilution Buffer VP**, 30 ml, ready for use. **Please shake before use!** Use 50 µl pro well in the assay.
4.	BUF PP	**Sample Buffer PP**, 120 ml, ready for use, yellow colored, please use for the reconstitution of **Standards (A-E)** and **Controls** and for dilution of **Samples** and **Controls.** **Please shake before use!**
5.	Control	**Control Sera KS1 and KS2**, 250 µl, lyophilised, contain human Serum and should be **reconstituted in each 250 µl Sample Buffer PP**. The IGFBP-3 target values and the respective ranges are given on the vial labels. The dilution should be according to the dilution of the respected samples. Use 50 µl pro well in the assay.
6.	Ab CONJ	**Antibody-HRP-Conjugate AK**, **12 ml, ready for use**, contains a mixture of biotinylated anti-human IGFBP-3 Antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. Use 100 µl pro well in the assay.
7.	WASHBUF 20x	**Washing Buffer (WP), 50 ml, 20X 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). Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
8.	SUBST	**Substrate (S),** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
9.	H_2SO_4	**Stopping Solution (SL),** 12 ml, ready for use, 0.2 M sulphuric acid, Caution acid!
10.		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

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

TECHNICAL NOTES

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.

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

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.

Incubation at room temperature means: 20-25°C

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must become adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/or false values, excessive shaking may result in high optical densities and/or false values.

Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided washing buffer diluted to usage concentration. Washing volume per washing cycle and well must be 300 µl at least.

The danger of handling with potentially infectious material must be taken into account.

When using an automatic microtiter plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamicly swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Standards and Controls

For the reconstitution of the lyophilised components (Standards A - E and Control Sera KS1 & KS2) the kit 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

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. The Mediagnost GmbH is not liable for any loss or harm caused by non-observance of the instructions, as far as no law withstands.

Temperature WILL affect the absorbance readings of the assay. However, values for the patient 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 patient's 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.

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.

ASSAY PROCEDURE

NOTES: All determinations (Standards, 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 Standards, Control Sera and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, the **Antibody-POD-Conjugate AK**, **the Substrate Solution S** as well as the **Stop Solution SL** should be added to the plate in the same order and in the same time interval each, respectively.

1) Please pipette on before in **all needed wells 50 µl Dilution Buffer VP**.
2) Add **50 µl Sample Buffer PP** in positions A1/2.
3) Pipette in positions B1/2 **50 µl** each **Standard A (0.4 ng/ml)**,
   pipette in positions C1/2 **50 µl** each **Standard B (2 ng/ml)**,
   pipette in positions D1/2 **50 µl** each **Standard C (6 ng/ml)**,
   pipette in positions E1/2 **50 µl** each **Standard D (15 ng/ml)**,
   pipette in positions F1/2 **50 µl** each **Standard E (30 ng/ml)**.
   To control the correct accomplishment **50 µl** of the 1:505 (or in respective dilution rate of the sample) in Sample Buffer **PP** diluted **Control Sera KS1 and KS2** can be pipetted in positions G1/2 and H1/H2.

Pipette **50 µl each** of the **diluted sample** (generally 1:505 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** (shake at 350 rpm).
5) After incubation aspirate the contents of the wells and wash the wells **5 times** with **300 µl Washing Buffer WP**.
6) Following the last washing step pipette **100 µl** of the **Antibody-POD-Conjugate AK** in each well.
7) Cover the wells with the sealing tape and incubate **1 hour** at **room temperature** (shake at 350 rpm).
8) After incubation wash the wells **5 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).

CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.20 and the absorbance of standard E should be greater than 1.00.

Samples, which yield higher absorbance values than **Standard E**, are beyond the standard curve, for reliable determinations such samples should be retested at a higher dilution.

Establishing the Standard Curve

The standards provided contain the following concentrations of hIGFBP-3

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	0.4	2	6	15	30

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 because the curve is in general (without respective transformation) not ideally described by linear regression. **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-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 6 ng/ml, Sample was 1:505 diluted: 6 x 505=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 (see Appendix). A graphic presentation is shown in Fig.4 and 5. It is recommended for each laboratory to establish its own normal range.

APPENDIX

Table 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.

Altersgruppe / Age group	Percentiles													
	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
7-9 y. 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
9-11 y. 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 y. 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
11-13 y. 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
13-15 y. 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
15-17 y. 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
70-80 y	**1.20**	1.50	**1.81**	2.00	**2.27**	2.47	**2.67**	2.87	**3.08**	3.32	**3.62**	4.09	**4.52**	5.44
> 80 y	**1.13**	1.43	**1.73**	1.92	**2.19**	2.39	**2.59**	2.79	**3.00**	3.23	**3.54**	4.00	**4.44**	5.36

Serum levels are given as mg/L
y. = years;
Determined with IGFBP-3 RIA (Blum et al. 1990)
The values above 70 years are extrapolated.

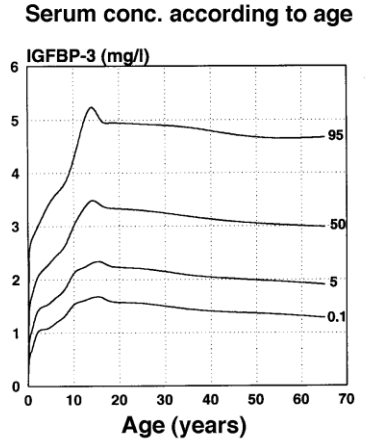

Fig. 5: Age-dependant normal values of IGFBP-3 (presented as 0.1., 5., 50., and 95. percentile)

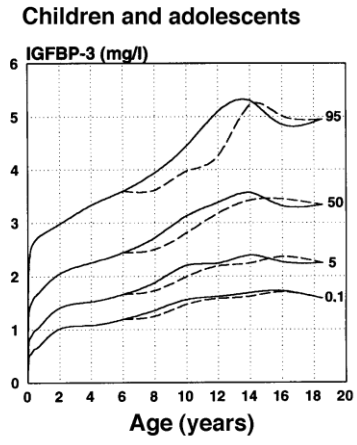

Fig. 6: Normal values of children and adolescents (girls —— boys - - - -)

LITREATURE

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SUMMARY – IGFBP – 3 total ELISA RMEE03A

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in **Sample Buffer PP** (yellow)	**1 ml each**
Control Serum KS1 & KS2	Reconstitution in **Sample Buffer PP** (yellow)	**250 µl 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 Sera KS1 & KS2: 1:505 in Sample Buffer PP (yellow colored), **mix directly and use within max. 60 min.** Use **50 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
50 µl	Dilution Buffer **VP**	Pipette **in <u>all</u> required number of wells**
50 µl	Sample Buffer **PP** as Blank	A1 and A2
50 µl	Standard **A** (**0.4 ng/ml**)	B1 and B2
50 µl	Standard **B** (**2 ng/ml**)	C1 and C2
50 µl	Standard **C** (**6 ng/ml**)	D1 and D2
50 µl	Standard **D** (**15 ng/ml**)	E1 and E2
50 µl	Standard **E** (**30 ng/ml**)	F1 and F2
50 µl	Control Serum **KS1**	G1 and G2
50 µl	Control Serum **KS2**	H1 and G2
50 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 (shake at 350 rpm)

5x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP**/**well**	each well
100 µl	Antibody-POD**-**Conjugate **AK**	each well

Incubation: 1 h at RT (shake at 350 rpm)

5x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 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)		

CAL A-E	A -E	Rec in 1 ml PP		
Control	KS1&KS2	Rec in 250 µl PP		
WASHBUF 20x	WP		1:20 DILU	A. dest.

Control		1:505 DILU	PP
SPE		1:505 DILU	PP
°C 20-25 °C			

50 µl	VP	A1 - End
50 µl	PP	A1/2
50 µl	CAL A (0.4 ng/ml)	B1/2
50 µl	CAL B (2 ng/ml)	C1/2
50 µl	CAL C (6 ng/ml)	D1/2
50 µl	CAL D (15 ng/ml)	E1/2
50 µl	CAL E (30 ng/ml)	F1/2
50 µl	CONTROL KS1 1:505 DILU PP	G1/2
50 µl	CONTROL KS2 1:505 DILU PP	H1/2
50 µl	SPE 1:505 DILU PP	
	TAPE	

1 h °C 20-25 ≥ 350 rpm ↔

5x 250 µl	5x WASHBUF WP
100 µl	AbCONJ AK
	TAPE

1h °C 20-25 ≥ 350 rpm ↔

5x 250 µl	5x WASHBUF WP
100 µl	SUBST TMB S

0.5 h °C 20-25

100 µl	H_2SO_4 SL
	MEASURE

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