IGFBP-3 RIA-CT

Radioimmunoassay with Coated Tubes
for Quantitative Measurement of

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

35.6-46.4°F

X **100 tubes**

h **IGF-R11**

mediagnost®
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DIN EN ISO 15223-1


Expiry date


Consider instructions for use

Lot-Batch Number


Manufactured by

Catalogue Number


Store at between


Contains sufficient for x tests


Radioactive

Table of contents

IGFBP-3 RIA CT, IGF-R11	100 Determinations with coated tubes
RUO	For Research Use Only!
Principle of the test	Radioimmunoassay
Duration (incubation period)	overnight (³ 15h)
Tracer	Iodinated native human IGFBP-3, < 55 kBq
Antibodies	specific, high-affinity polyclonal antiserum
Cross reactivity with IGFBP-1,-2,-4,-5,-6	< 0.3%
Buffer	2fold concentrate
Standard	5 single standards: 1 – 81 µg/L, native human Serum
Assay Range	0.8 – 24000 µg/L
Control	2 control sera, freeze-dried – RilibäK conform
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:301
Analytical sensitivity	Ø< 0.8 µg/L
Intra- / Interassay Variance	Ø < 10 %
Half Maximal displacement	in the range 12 µg/L

1 INTENDED USE

This radioimmunoassay kit is intended to be used for research only. It quantifies human IGFBP-3 in serum, plasma, or other human biological fluids (e.g. follicular fluid, seminal plasma).

2 INTRODUCTION

Insulin-like growth factors (IGF)-I and -II are bound to specific binding proteins (IGFBPs) in the circulation. Until now at least seven binding proteins have been identified and classified as IGFBP-1 to -7 (1, 2).
Insulin-like growth factors (IGF)-I and -II are bound to specific binding proteins (IGFBPs) in the circulation. Until now at least seven binding proteins have been identified and classified as IGFBP-1 to -7 (1, 2). These binding proteins are expressed by different kinds of tissues and show specific posttranslational modifications like glycosylation or phosphorylation. Their main function is – together with specific proteases - the regulation of the IGF bioavailability. The most frequent IGFBP in blood is IGFBP-3. Most IGFBP-3 in the circulation is bound in a ternary complex formed by IGF-I or -II, IGFBP-3 and the so called acid-labile subunit (ALS, 3-5). Most of the IGFBP-3 in blood 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 radioimmunoassays for IGFBP-3, able to detect IGFBP-3 within the ternary complex, provided new in-sights into its 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).

3 ASSAY PRINCIPLE

The Mediagnost IGF-R11 is a competitive radioimmunoassay, based on a specific, highly affine, polyclonal antiserum, resulting from an immunization of rabbits with isolated native human IGFBP-3. The antiserum is able to detect IGFBP-3 quantitative within the ternary complex. The test is competitive which means that the IGFBP-3 in the unknown sample and the standards competes with the radioactively labelled IGFBP-3 (I^{125}) of the tracer for binding to the antiserum. The assay is incubated in Streptavidin-coated tubes. The capture antibody mediates the binding between the specific antibody and the tubes, making it easy to wash out the unbound tracer after the incubation.
The amount of the bound tracer is determined by measuring the radioactivity, the lower the measured signal, the higher is the IGFBP-3 content in the sample or standard. The IGFBP-3 concentrations in the samples are quantified by comparison with a standard containing a known amount of native human IGFBP-3.

4 WARNINGS AND PRECAUTIONS

- **For In Vitro and Research Use. Not for use in diagnostic procedures.**
- For professional use only.
- Before starting the assay, read the instructions completely and carefully, follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood.
- The acquisition, possession and use of the kit are subject to the regulations of the national nuclear regulatory authorities.
- Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
- 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.
- Reagents contain sodium azide as preservative, however, highly diluted (0.02%).
- Safety Data Sheet available on request.
- Samples, standards and controls should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the test-protocol are recommended.
- Do not use obvious damaged or microbial contaminated or spilled material.
- **Caution:** This kit contains material of human and/or animal origin. Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibodies. No known methods can offer total assurance of the absence of infectious agents; therefore all components and specimens should be treated as potentially infectious.

4.1 Radioactivity

Before ordering or using radioactive materials, it is necessary to take the appropriate actions to ensure compliance with national regulations governing their use. Local rules in each establishment, which define actions and behavior in the radioactivity working areas, should also be adhered to. The advice given here does not replace any local rules, instructions or training in the establishment, or advice from the radiation protection advisers. It is important to follow the code of good laboratory practice in addition to the specific precautions relating to the radionuclide Iodine-125 used.

Iodine-125 has a radioactive half-life T1/2 of 60 days and emits 35.5 keV gamma radiation, 27 – 32 keV x-rays and no beta radiation. Shielding is effective done by lead, first half value layer is 0.07 mm lead, reduction to 10 % is made by 0.2 mm. To reduce the radiation dose time spent handling radioactivity should be minimized (plan ahead), and distance from source of radiation should be maximized (doubling the distance from the source quarters the radiation dose).

Formation of aerosols, e.g. by improper opening and mixing of vials or pipetting of solutions which may cause minute droplets of radioactivity become airborne, is a hazard and should be avoided.

Solutions containing Iodine should not be made acidic, because this might lead to the formation of volatile elemental Iodine. As some iodo-compounds can penetrate rubber gloves, it is advisable to wear two pairs of gloves, or polyethylene gloves over rubber.

For cleaning of contaminated areas or equipment, the Iodine-125 should be rendered chemically stable by using alkaline sodium thiosulphate solution together with paper or cellulose tissue.

4.2 General First Aid Procedures:

- *Skin contact:* Wash affected area thoroughly with water at least 15 minutes. Discard contaminated cloths and shoes. See a physician.
- *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. See a physician.
- *Ingestion:* If swallowed, wash out mouth thoroughly with water, provided that the person is conscious. Immediately see a physician.
- The handling of radioactive and potentially infectious material must comply with the following guidelines:
- The material should be stored and used in a special designated area.
- Do not eat, drink or smoke in these areas.
- Never pipette the materials with the mouth.
- Avoid direct contact with these materials by wearing laboratory coats and disposable gloves.
- Spilled material must be wiped off immediately. Clean contaminated areas and equipment with a suitable detergent.
- Unused radioactive material and radioactive waste should be disposed according to the recommendations of the national regulatory authorities.

5 SAMPLES

5.1 Sample Type

Serum, plasma, breast milk, follicular fluid, seminal plasma, urine and cerebrospinal fluid. Serum and Heparin/EDTA/Citrate Plasma yield comparable values.

5.2 Specimen collections

Use standard venipuncture for the blood sampling. Haemolytic reactions and icteric or lipaemic samples are to be avoided.

5.3 Required sample volume

10 µL

5.4 Sample stability

- Storage at 68-77°F (20-25°C): max. 3 days
- Storage at -4°F (-20°C): min. 2 years
- Freezer /-thaw cycles: max. 10

The storage of samples over a period of 2 years at -4°F (-20°C) showed no significant influence on the reading. Freezing and thawing of samples should be minimized. 10 Freezing-/ Thawing showed no significant effect on samples.
IGFBP-3 levels remain stable up to elevated temperatures in normal situations, if undiluted. If diluted, however, stability is extremely decreased (see chapter 5.6 for necessary precautions).

5.5 Interference

If the samples are diluted as recommended, either triglycerides, bilirubin, haemoglobin nor biotin exert no significant influence up to concentrations of 100 g/L, 200 mg/L, 5 g/L and 3 µg/mL respectively on the measurement of IGFBP-3 in human serum.

5.6 Sample dilution

- **Recommended** Dilution: **1:301** with Assay Buffer **DB**
  Example: **10 µL** sample added to **3 mL** Assay Buffer **DB** (301 dilution factor) and mixed immediately.
- Depending on the expected IGFBP-3 values the dilution can vary from **1:100 to1:400**
- **Important:** Because IGFBP-3 is not stable in diluted solutions, please use only **chilled, preferably ice-cold** Assay Buffer **DB**. Mix immediately after addition of the samples into Assay Buffer **DB.** The time interval between the sample dilution and incubation should be as short as possible, i.e. the diluted samples should be processed fast as can.
- In the other human liquids listed above, the IGFBP-3 values can vary considerably; the optimum dilution must be determined by the customer
- Dilution of Controls **M** and **N** according to dilution of samples.

6 MATERIALS

6.1 Reagents provided

The reagents and coated tubes listed below are sufficient for 100 determinations including the standard curve.

2xDB	**Dilution Buffer,** 2-fold concentrated blue coloured	**1 x 125 mL**
R	**Capture Antibody,** anti-rabbit IgG, biotin-conjugated lyophilized	**1 x 5.5 mL**
S	**Specific Antibody,** rabbit-anti-hIGFBP-3, lyophilized, blue coloured	**1 x 5.5 mL**
C	**Tracer ^{125}I-IGFBP-3,** lyophilized, **< 1.5 µCi or < 55 kBq**, red coloured	**1 x 11 mL**
F - J	**Standards,** lyophilized, (native human IGFBP-3) Concentrations given on vial-labels	**5 x 500 µL**
M	**High Control,** lyophilized (human serum): Concentration see certificate	**1 x 100 µL**
N	**Low Control,** lyophilized (human serum): Concentration see certificate	**1 x 100 µL**
T	**Tubes** coated with streptavidin	**100 tubes**
	**Instructions for use**	**1 x**
	Quality Certificate	**1 x**

6.2 Reagents required, but not provided

- Cold demineralised water or distilled water (Aqua destillata) **(A. dest.), 125 mL**
- Graduated cylinder, Pipettes: 10 mL, 500 µL, 100 µL and 10 µL; 50 µL, 100 µL and 500 µL repeating pipettes (Multi Step) are recommended.
- Vortex mixer
- Shaking device is recommended
- Device for aspiration of liquid supernatant is recommended
- Gamma counter

7 TECHNICAL NOTES

7.1 Storage Conditions

Store the kit at **35.6-46.4°F (2-8°C)** after receipt until its expiry date. The lyophilized reagents should be stored at **-4°F (-20°C) after reconstitution**. Avoid repeated thawing and freezing. The shelf-life of the **reagents after opening** is in accordance with the Tracer **C** shelf life.

7.2 Reagent Preparation

Ensure that lyophilized materials are completely dissolved on reconstitution. It is recommended to touch the tubes with lyophilized material once on a solid base before first opening in order to accumulate the material at the bottom of the tubes. After addition of the Assay Buffer **DB** it is recommended to keep the reconstituted reagents at **68-77°F (20-25°C)** for half an hour and then to mix the vigorously with a Vortex mixer. This is important in particular for the Controls **M** and **N.**
Possible residues on the coated tubes **T** are unavoidable for production reasons - the function is not impaired.

2xDB	125 mL fill up to **250 mL** with cold A. dest. (→ Assay Buffer **DB**)
C	Reconstitute with **11 mL** Assay Buffer **DB**
R	Reconstitute with **5.5 mL** Assay Buffer **DB**
S	Reconstitute with **5.5 mL** Assay Buffer **DB**
F - J	Reconstitute with **500 µL** Assay Buffer **DB** each.
M and N	Reconstitute with **100 µL** Assay Buffer **DB** each. Ensure that lyophilized materials are completely dissolved on reconstitution. Dilution according to sample dilution with Assay Buffer **DB (e.g. 1:301)**

Samples (standards, controls and specimen) should be **determined in duplicate**. For optimal results, accurate pipetting and adherence to the protocol are recommended.

8 ASSAY PROCEDURE

Samples (standards and specimens) should be determined in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

Flow Chart of Assay Protocol

Nr.	Tubes	DB F-J, M-N Samples	R	S	C
1,2	TC	---	---	---	100
3,4	NSB	**DB:** 150	50	---	100
5,6	B_0	**DB**: 100	50	50	100
7-16	Standards	**F-J**: 100	50	50	100
17,18	High Control	**M**: 100	50	50	100
19,20	Low Control	**N**: 100	50	50	100
21,22	Sample 1	100	50	50	100
23,24	Sample 2	100	50	50	100
etc.					

All volumes are given in µL.

1) Labelling of assay tubes should be done in the following order:
1, 2 total counts **(TC)**
3, 4 non-specific binding **(NSB)**
5, 6 **Assay Buffer DB (zero standard, B_0)**
7 - 16 duplicates of **Standards (F – J)**
17, 18 **High Control M**
19, 20 **Low Control N**
from 21 duplicates of **Samples**.

2) Add **150 µL** of **Assay Buffer (DB)** to tubes 3 and 4.

3) Add **100 µL** of **Assay Buffer (DB) as zero standard, B_0** to tubes 5 and 6

4) Add **100 µL** of **Standards** (**F – J)** to tubes 7-16
7, 8 standard F
9, 10 standard G
11, 12 standard H; etc.

5) Add **100 µL** of diluted **High Control (M)** to tubes 17 and 18.

6) Add **100 µL** of diluted **Low Control (N)** to tubes 19 and 20.

7) Add **100 µL** of diluted **Sample** to tubes 21 and 22, etc.

8) Add **50 µL Capture Antibody (R)** beginning with tube 3.

9) Add **50 µL Specific Antibody (S)** beginning with tube 5.
-All solutions are coloured **blue**!-

10) Add **100 µL Tracer (C)** to all tubes.

Mark tubes 1 and 2 (total counts), seal with a stopper or remove until step 15.
-All solutions are coloured **violet**!-

11) Incubation conditions: **overnight** (at least 15 hours) **at room temperature** on a shaking device at **350 rpm**. Without shaking device, the tubes must be mixed thoroughly

by a vortex mixer. Then incubate also overnight at room temperature (with slightly reduced binding), or, for **2 days** (or the weekend) **at 35.6 -46.4°F (2 - 8°C)**.

12) Decant or aspirate the liquid (except tubes 1 and 2 !) completely. Take care that the coating of the tubes remains intact.

13) Add **500 µL** of **Assay Buffer (DB)** to the tubes (except tubes 1 and 2 !).

14) Decant or aspirate the liquid (see step 12).

15) Count the radioactivity of all tubes in Gamma Counter for **1 to 3 min**.

Alternative Pipetting Schema for working steps 8 and 9

Mix the reconstituted **Reagents S** (**Spec. Antibody) and R (Capture Antibody)** externally (1:1), add 100 µL of this mix beginning with the tube 3.

With this variant a determination of the Nonspecific Binding NSB is no longer possible, please take the lack of the NSB into consideration when planning the assay protocol and when evaluating the test.

9 QUALITY CONTROL

All kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated.

10 EVALUATION OF RESULTS

10.1 Establishing of the Standard Curve

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

Standard	F	G	H	I	J
ng/mL	1	3	9	27	81

1) Calculate the average counts (AC) of each pair of tubes. This gives the values for B.
2) Subtract the average counts (AC) of NSB tubes (3 and 4) from the mean counts of the standards, controls and samples. This gives the corrected values for B.
3) The corrected value from tubes 5 and 6 (Assay Buffer) is B0.
4) Calculate the percent bound %B/B0 by dividing the corrected B-values by B0: %B/B0 = B/B0 x 100%.
5) Plot %B/B0 versus the standard concentrations on either semi-logarithmic or logit-log paper or evaluate by using a computer program.

10.2 Quality Criteria

Non-specific Binding:	**specific** Binding:
Quality Criteria: **%NSB/TC < 5%**	Quality Criteria: **%B0/TC > 15%**
Example of calculation with the exemplary data see below:	
NSB / TC x 100%	(B0 - NSB) / TC x 100%
335.5 cpm / 18832.4 cpm x 100% = 1.8% NSB/TC < 5%	(7876.5 cpm – 335.5 cpm) / 18832.4 cpm x 100% = 40.0% B0/TC > 15%

10.3 Example of Typical Standard Curve

The exemplary data and the standard curve in Figure 1 **cannot** be used for the calculation of the test results. You have to establish a standard curve for each test you conduct.

Example	TC	NSB	B0	F	G	H	I	J
cpm	18832.4	335.5	7876.5	7130.2	5562.9	3252.2	1631.2	860.3
%B/B0	-	-	-	90.10	69.32	38.68	17.18	6.96
ng/mL	-	-	-	1	3	9	27	81

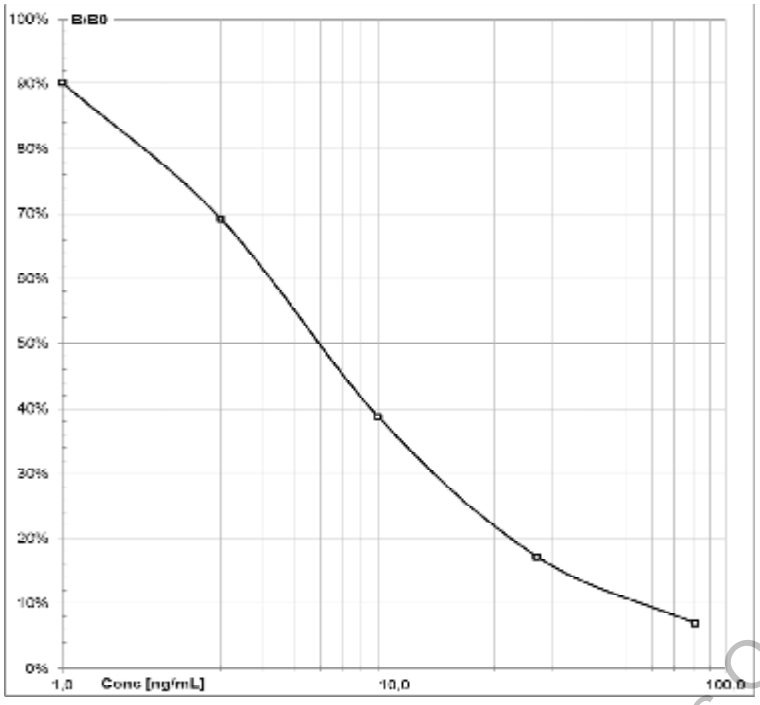

Figure 1 Examplary Standard Curve

10.4 Evaluation of sample concentrations

Read the concentration value (abscissa) corresponding to the %B/B0 of the sample as in the example given below :

Average counts of **NSB**	**335.5 cpm**
Average counts of zero Standard **B0**	**7876.5 cpm**
Average counts of Sample:	**3074.6 cpm**

%**B/B0** = (**sample counts** - **NSB**) / (**B0** - **NSB**) **x 100**
= **(3074.6 – 335.5) / (7876.5 – 335.5) x 100%**
= 0.3632 x 100%
= 36.32%

A 36.32%-value on the y-axis (ordinate) results in 9.922 ng/mL on the x-axis (abscissa).
Multiply the concentration value determined graphically or by the aid of a computer program with the dilution factor (e.g.: 301)
Example: 9.922 ng/mL x 301 = 2987 ng/mL or 2.99 mg/L respectively.

11 LIMITATIONS OF PROCEDURE

Sensitivity and specificity are 97 and 94% respectively, if the 5th percentile is used as cut-off value (9, page 156). A number of factors influence plasma concentration of IGF-I and/or IGFBP-3 and should be taken into account for appropriate interpretation.
Basically, the result of immunological test systems can be affected by various sample components such as medications or lipids. Their influence is reduced by the assay design, but cannot be excluded completely.

12 EXEMPLARY VALUES

IGFBP-3 levels are strongly age-dependent in children, less so in adults. Exemplary IGFBP-3 concentrations are given for various age-groups in Table 1 by the percentiles.

Tab. 1 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.

		Percentiles													
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 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
	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
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**	5.50
> 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**	5.45

Serum levels are given as mg/L

Determined with IGFBP-3 RIA (Blum et al. 1990)
The values above 70 years are extrapolated.

13 PERFORMANCE CHARACTERISTICS

13.1 Sensitivity

Sensitivity was assessed by measuring the B0 16 times in one assay; 2-fold standard deviation was calculated and subtracted from mean B0. The concentration of the resulting %B/B0 was recalculated. The results show analytical sensitivity of < 0.8 µg/L in the IGF-R11.

13.2 Specificity

Specificity of the test system was evaluated by investigating the influence of homologue proteins: other IGFBPs. It was shown that none of the tested IGFBPs interfered significantly with the antibodies used (Table 2).

Tab. 2 Specificity : Cross-reactivity with homologous IGFBPs. Recombinant IGFBPs were added in dilution buffer and the signal intensity was measured in the Mediagnost IGFBP-3 RIA-CT, IGF-R11.

	recombinant IGFBP [ng/mL]	measured IGFBP-3 [ng/mL]	Cross Reactivity [%]
IGFBP-1	1000	1.128	0.11
IGFBP-2	1000	0	0
IGFBP-4	1000	0	0
IGFBP-5	330	0.88	0.27
IGFBP-6	1000	0.022	0.00

13.3 Precision

Intra-Assay Variance

Four samples have been measured 5 times in the same assay. The results are shown in Table 3. The measured coefficient of variation (CV) for the exemplarily shown four samples is on average.

Tab. 3 Intra-Assay Variation

	IGFBP-3 [ng/mL]					Mean [ng/mL]	Standard-deviation [ng/mL]	CV [%]	Number [n]
Sample 1	3921	3988	3921	3855	3844	3906	58.35	1.49	5
Sample 2	1307	1250	1198	1268	1285	1262	41.32	3.28	5
Sample 3	2993	2837	2965	2693	2965	2891	125.97	4.36	5
Sample 4	4541	4515	4255	4292	4342	4389	130.91	2.98	5

Inter-Assay Variance

Serum samples where measured in independent assays. On average the coefficient of variation was < 10%. Results are shown in detail in Table 4.

Tab. 4 Inter-Assay Variation. Six samples have been tested in a period of three years for several times in different lots. The mean coefficient of variation was 5.52%, measured in a period of 3 years.

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Mean	[ng/mL]	1690.83	1628.50	3350.48	3512.23	3097.57	3071.89
Standard Deviation	[ng/mL]	111.72	108.40	185.04	161.37	147.82	151.90
CV	[%]	6.61	6.66	5.52	4.59	4.77	4.94
Number	[n]	68	70	47	35	23	22

13.4 Linearity

Linearity of the IGF-R11 was tested by serial dilution of different serum samples and measurement of the IGFBP-3 concentration. Results are shown in Figure 2. Samples can be diluted in a broad range according the requirements of the experimental setting. We recommend a standard dilution of 1:301 (0.00332).

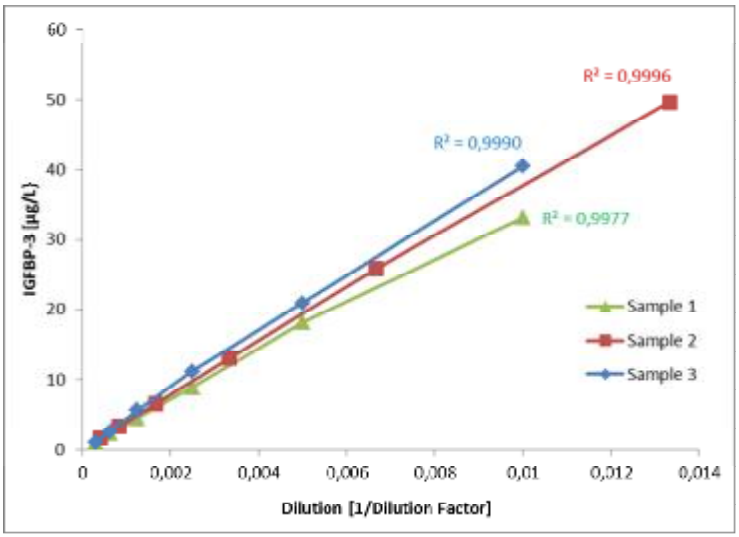

Figure 2 Linearity of sample dilution. Three samples with different amount of IGFBP-3 were diluted and IGFBP-3 concentration was measured.

13.5 Interference

Assessment of the influence of hemolytic, icteric and lipaemic samples was done by an artificial system. Exemplarily, five human serum samples were enriched with triglycerides (up to 100 g/L), bilirubin (up to 200 mg/L) and hemoglobin (up to 5 g/L) and IGFBP-3 was measured in the enriched and not enriched samples. Testing of biotin interference was done with three serum samples enriched with different concentrations of biotin up to 3000 ng/mL. No significant influence of these potentially interfering substances was detected (Table 5). But this might depend on the individual sample and thus hemolytic, icteric and lipaemic samples should be avoided.

Tab. 5 IGFBP-3 measurements in artificially enriched samples. Shown is the relative recovery of IGFBP-3 in comparison to the non-enriched sample

	Triglyceride 100 mg/mL	Bilirubin 200 µg/mL	Hemoglobin 1 mg/mL	Hemoglobin 5 mg/mL	Biotin 3000 ng/mL
Serum 1	104	90	83	-	111
Serum 2	97	88	108	-	111
Serum 3	79	89	129	105	103
Serum 4	93	97	137	109	-
Serum 5	-	100	128	103	-

13.6 Trueness/ Assay Calibration

As the assay was developed and characterized no international standard material was available. Thus, serum of healthy blood donors was pooled and IGFBP-3 concentration measured. This original calibration was used for the evaluation of reference values (Blum et al. 1990). Because of analyte instability and differential glycosylation, a valid reference material for immunological assay is still not available (2016).

Means to prove the trueness and the specificity of the Mediagnost IGFBP-3 RIA-CT, IGF-R11 are the comparison with other test systems as well as the results of external quality assessment schemes. Both approaches were used to prove the validity of the Mediagnost IGFBP-3 RIA-CT.

13.7 External Quality Assesment

Mediagnost and some of Mediagnost customers take part in the External Quality Control (EQA) of the Reference Institute of Bioanalytics. In Figure 3 the results of the EQA from 2010 to 2016 are shown. The mean deviations of the method-specific target value are 3.25% / 4.88% and for the total, method-independent target value -10.39% / -6.90% for sample A and B, respectively.

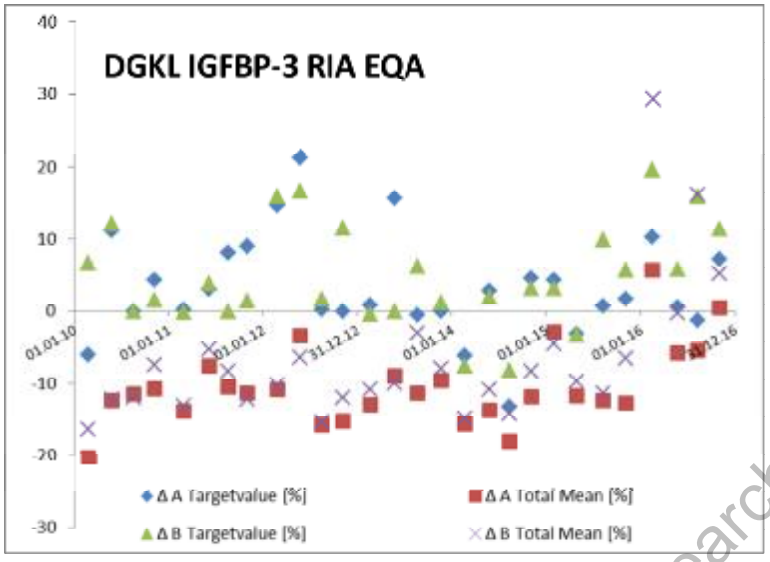

Figure 3 Results of EQA. In each quarter of the year IGFBP-3 is measured in two samples provided by an independent institution, which also analyses the test results. Here the relative deviations of the method-specific target values and the method independent total mean values are shown for the samples A and B in %.

14 ASSAY COMPARISON

Mediagnost IGF-R11 was compared with immunoassays of two competitors and an in-house test system of a university hospital.
The comparison of the measured values from IGF-R11 and the other assays by linear regression results in below regression equations:

$y = 1.0851x – 0.5397;\ R^2 = 0.9662;\ n = 87$
$y = 1.1931x + 0.0156;\ R^2 = 0.9275;\ n = 57$
$y = 0.8615x + 282.17;\ R^2 = 0.7151;\ n = 59$

These data prove that Mediagnost IGFBP-3 RIA-CT, IGF-R11, correlates well with the other IGFBP-3 assays.

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16 ASSAY PROCEDURE

Reagent preparation:		Reconstitution:	Dilution:
2xDB	**Dilution Buffer**	-	Before use dilute **1:2** with cold **A. dest.** → Assay Buffer **DB**
C	**Tracer**	in **11 mL** Assay Buffer **DB**	–
R	**Capture Antibody**	in **5.5 mL** Assay Buffer **DB**	–
S	**Specific Antibody**	in **5.5 mL** Assay Buffer **DB**	–
F-J	**Standards**	in **500 µL** Assay Buffer **DB each**	–
M+N	**Controls**	in **100 µL** Assay Buffer **DB each**	**1:301** with ice-cold Assay Buffer DB
Keep the reconstituted reagents at **68-77°F** (**20-25°C)** for approx. **30 min** and then mix vigorously with a Vortex mixer. This is important in particular for the Controls **M** and **N.**			–
Dilute Sample with **ice-cold** Assay Buffer **DB** e.g. **1:301, mix directly** + process as fast as can.			

Assay Procedure for Double Determinations

Nr. of Tubes	Contents of Tubes	DB, F-J M, N Samples	R (Capture Antibody)	S (Specific Antibody)	C (Tracer)
1 / 2	Total Counts **TC**	–	–	–	100 µL
3 / 4	Assay Buffer DB = Non-specific binding **NSB**	150 µL **DB**	50 µL	–	100 µL
5 / 6	Assay Buffer DB = Zero Standard **B0**	100 µL **DB**	50 µL	50 µL	100 µL
7 - 16	Standards	100 µL **F-J**	50 µL	50 µL	100 µL
17 / 18	Diluted high Control	100 µL **M**	50 µL	50 µL	100 µL
19 / 20	Diluted low Control	100 µL **N**	50 µL	50 µL	100 µL
21 / 22	Diluted Sample 1	100 µL	50 µL	50 µL	100 µL
23 / 24	Diluted Sample 2	100 µL	50 µL	50 µL	100 µL
etc.					
Coloration after addition		**coloration light blue**	**coloration light blue**	**coloration deeper blue**	**coloration violet**

Nr. 1, 2 remove until counting the activity.
Incubation, overnight at 68-77°F (20-25°C), at least 15 hours on a shaking device at 350 rpm. ***Alternative pipetting without shaking device, the tubes must be mixed thoroughly by a vortex mixer. Then incubate also overnight at room temperature (with reduced binding), or for 2 days (e.g. over the weekend) at 35.6-46.4°F (2-8°C)***
Decant or aspirate the liquid completely. Take care that the coating of the tubes remains intact.
Add **500 µL** of Assay Buffer **DB** for washing to the tubes
Decant or aspirate the liquid completely (see above)
Count the **activity** of all tubes with a Gamma Counter.