IGF-I RIA

Radioimmunoassay for Quantitative Determination of

Insulin-like Growth Factor I (IGF-I)
(IGFBP-blocked)

For Research Use Only

For Research Use Only.
Not for use in diagnostic procedures.

+2°C +8°C

for 100 Tubes

REF **R20**

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Symbols 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

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Information for Use

IGF-I RIA, R20	100 Determinations
Principle of the test	Non - extractive radioimmunoassay
Duration (incubation period)	2 days + 1.5 h
Tracer	Iodinated recombinant IGF-I, < 55 kBq
Antibodies	specific, high-affinity polyclonal antiserum
Cross reactivity with IGF-II	< 0.103 %
Buffer	Ready to use
Reference material	International Standard WHO/NIBSC 02/254
Calibrator	8 single calibrators: 0 – 10 ng/mL, recombinant IGF-I
Assay Range	0.064 – 1010 ng/mL
Control	2 control sera, freeze-dried - RiliBäK conform
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:101
Analytical sensitivity	ø 0.064 ng/mL
Intra- / Interassay Variance	ø 4.76 / 5.06 %
Half Maximal displacement	at < 3.5 ng/mL

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1 INTENDED USE

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

2 INTRODUCTION

Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation, differentiation and specific functions of many cell types (1-3). IGF-I is identical with Somatomedin C (Sm-C) (4) and has a molecular weight of 7649 daltons (5). Its major regulators are growth hormone (GH) and nutrition (6), although its production in specific tissues is affected by a multitude of tropic hormones and other peptide growth factors. In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven classes of IGFBPs which are known at present (7,8,22) either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II (9,10).

A major problem of IGF-I measurement results from the interference of IGFBPs in the assay. Direct determinations in untreated serum samples (11) give false values because of the extremely slow dissociation of the IGF-I/IGFBP-3 complexes during the assay incubation. Depending on the ratio IGF-I to IGFBP the following errors may occur:

-in samples with low IGF-I concentration, IGFBP-complexation will take place predominantly with the IGF-I tracer, thus leading to false-high results in a competitive RIA. Effect: Overestimation of low IGF-I levels.

-in samples with high IGF-I concentration, unmarked IGF-I from the sample will be predominantly complexed by IGFBPs and therefore withdrawn from measurement. Effect: Underestimation of high IGF-I levels.

Therefore, various techniques were applied to physically separate IGF-I from its binding proteins before measurement, including (a) size exclusion chromatography under acidic conditions, (b) solid-phase extraction and (c) acid-ethanol extraction (2,12,13). These techniques, however, are either inconvenient or time-consuming or give incomplete and not-reproducible recoveries. The most widely used method is the acid-ethanol extraction (13,14) with a recovery of only 70-80 % of IGFBP-bound IGF-I as a result of co-precipitation. The absolute results of such an extraction are therefore false low (15). The extraction removes

the IGFBPs only insufficiently and leads to reduction in sensitivity of the assay due to pre-dilution of the samples by the extraction procedure. Furthermore, the remaining IGFBP may still interfere in the assay. In addition, the acid-ethanol extraction is ineffective in specimens other than serum or plasma (e.g. cell culture media), in which determination of IGF-I is already difficult enough due to the fact that IGFBPs are frequently present at large excess.
To avoid these difficulties, an uncomplicated assay was developed, in which special sample preparation is not required before measurement.

3 PRINCIPLE

In order to dissociate IGF-I from the IGFBPs, the samples must be diluted in an acidic buffer (Figure 1). The diluted samples are then pipetted into the assay tubes. The IGF-I antiserum containing an excess of IGF-II is dissolved in a buffer, which is able to neutralize the acidic samples. After the IGF-I antibody solution has neutralized the samples, the excess IGF-II occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of free IGF-I. With this method, the IGFBPs are not removed, but their function and therefore their interference in the assay is neutralized.

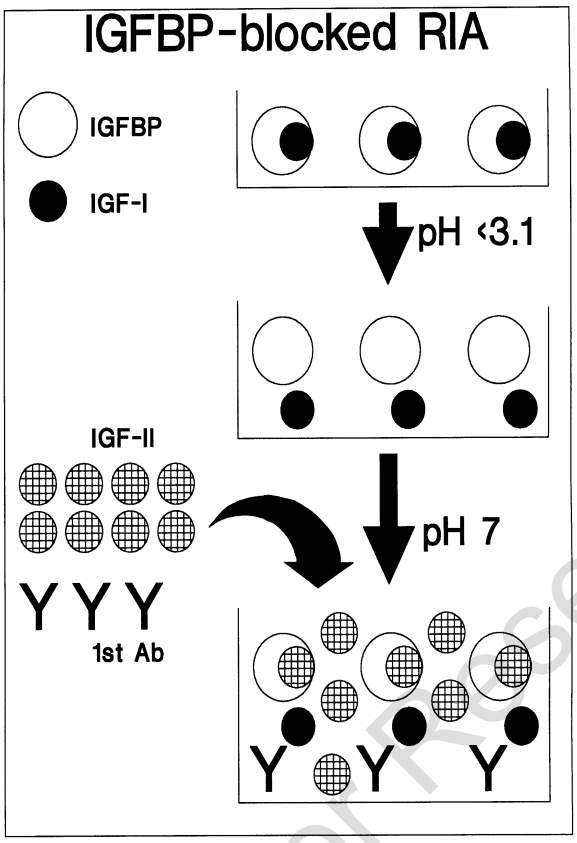

Figure 1 Principle of the IGFBP-blocked IGF-I RIA

Due to the extremely low cross-reactivity of the IGF-I antibody with IGF-II, excess IGF-II does not disturb the interaction of the first antibody with IGF-I or IGF-I tracer. The assay is then continued like a conventional RIA using a second antibody for the separation of bound and free tracer.
The colour of the solutions makes possible for every tube a control of the respective performance step. This enables you to check your pipette plan, if necessary. Dilution and acidification buffer (including the reconstituted calibrators and diluted samples too) are coloured in green through addition of a pH indicator dye. After addition of the uncoloured IGF-I antibody solution, the now neutralized solutions turn blue. Finally, addition of the red coloured tracer solution turns the entire incubation colour violet.

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4 WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For professional use only.
3. The acquisition, possession and use of the kit are subject to the regulations of the national nuclear regulatory authorities.
4. 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.
5. Before use, all kit components should be brought to room temperature at 20 - 25 °C (68 - 77 °F). Precipitates in buffers should be dissolved before use by thorough mixing and warming.
6. Do not mix reagents of different lots. Do not use expired reagents.
7. **Caution:** This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibody. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and specimens should be treated as potentially infectious.
8. Reagents contain Sodium-Azide (0.02 %) as preservative, however highly diluted. Safety Data Sheet available on request.
9. Do not use obvious damaged or microbial contaminated or spilled material.
10. Radioactivity - Radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories, or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the state commissioner of health, the Nuclear Regulatory Commission, or a state with which the Nuclear Regulatory Commission has entered into an agreement for the exercise of regulatory authority.
    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 I-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 0.02 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 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.

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.

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5 SAMPLES

5.1 Sample Type

Serum and Plasma

Serum and Heparin/EDTA Plasma yield comparable values.
The IGF-I levels are reduced in citrate plasma samples, because of the relatively high amount of anticoagulant.

Mediagnost R20 can be also used for measurement of matrices with low concentrations of IGF-I like saliva, cerebrospinal fluid, urine, breast milk and cell culture supernatant. Matrices other than serum and plasma cannot be diluted but acidified by adding Acidification Buffer **AB**.

5.2 Specimen collections

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided. Blood samples may be taken at any time of the day. Whole blood should be processed within a few hours and stored frozen at -20 °C (-4 °F) until measurement.

5.3 Required sample volume

10 µL

5.4 Sample stability

In firmly closable sample vials

- Storage at **20 - 25 °C** (68 - 77 °F): max. 6 days
- Storage at **-20 °C** (-4 °F): min. 2 years
- Freezer /-thaw cycles max. 3

The storage of samples over a period of 2 years at **-20 °C** (-4 °F), showed no influence on the measurement. Freezing and thawing of samples should be minimized. 3 Freezing-/ Thawing showed no effect on samples.

5.5 Interference

Either triglycerides, bilirubin nor hemoglobin exert any influence up to concentrations of 100 g/L, 200 mg/L, 5 g/L respectively on the measurement of IGF-I in human serum. Rec.IGFBP-3 don´t interfere with IGF-I measurement up to the concentration of 12 mg/L in Dilution Buffer **DB**.

5.6 Sample dilution

- **Dilution: 1:101** with Dilution Buffer **DB**
  Example: Add **10 µL** Sample to **1 mL Dilution Buffer DB** (101 dilution factor).
- The **serum and plasma samples** must be diluted at least **1:20 in Dilution Buffer DB.**

- Matrices **<u>other than serum and plasma</u>** must be acidified by adding **Acidification Buffer AB** (1/10th of the sample volume).
  Example: Add **20 µL Acidification Buffer AB** to **200 µL** sample (dilution factor: 1.1).

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6 MATERIALS

6.1 Reagents provided

The reagents listed below are sufficient for 100 tubes including the calibrator curve.

***AB**	**Acidification Buffer,** ready for use, coloured	**1 x 12.5 mL**
DB	**Dilution Buffer**, ready for use, coloured	**1 x 125 mL**
A	**Assay Buffer** ready for use	**1x 30 mL**
B	**1st Antibody,** lyophilized (anti-hIGF-I) contains rabbit IgG and rec. hIGF-II	**1 x 11 mL**
C	**Tracer: ^{125}I-IGF-I,** lyophilized, < 1.5 µCi or < 55 kBq - red coloured	**1 x 11 mL**
D	**Non-Specific Binding (NSB),** lyophilized, Rabbit immunoglobulin	**1 x 500 µL**
E - L	**Calibrators,** lyophilized, (rec. Human IGF-I) Concentrations given on vial-labels in ng/mL	**8 x 500 µL**
M	**Control High,** lyophilized (human serum): Concentration see certificate - lyophilized	**1 x 100 µL**
N	**Control Low,** lyophilized (human serum): Concentration see certificate - lyophilized	**1 x 100 µL**
O	**2nd Antibody,** lyophilized (anti-rabbit immunoglobulin)	**1 x 1 mL**
P	**Precipitation Reagent** ready for use after adding **O**	**1 x 55 mL**
	**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.)**
- Pipettes: 10 mL, 1 mL, 500 µL, 100 µL, 10 µL; 100 µL, 500 µL and 1 mL repeating pipettes are recommended.
- Disposable polystyrene or polypropylene tubes. Conical tubes are highly recommended because of the small immune precipitates. The use of round-bottom tubes may cause formation of insufficiently compact pellets.
- Vortex mixer
- Centrifuge
- Device for aspiration of liquid supernatant
- Gamma counter

*The IGF-I measurement in other matrices than serum or plasma is possible. The reagent: **Acidification Buffer AB** is included in the kit for these applications.

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7 TECHNICAL NOTES

7.1 Storage Conditions

Store the kit at **2 - 8 °C (35.6 - 46.4 °F)** after receipt until its expiry date. The lyophilized reagents should be stored at **-20 °C (-4 °F)** 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. It is recommended to keep the reconstituted reagents at **20 - 25 °C** (68 - 77 °F) for half an hour and then to mix them vigorously with a Vortex mixer. This is important in particular for the Controls **M** and **N**.

B	Reconstitute with **11 mL** Assay Buffer **A.**
C	Reconstitute with **11 mL** Assay Buffer **A.**
D	Reconstitute with **500 µL** Assay Buffer **A.**
E - L	Reconstitute with **500 µL** Dilution Buffer **DB.**
M	Reconstitute with **100 µL A. dest**. Further dilution according to sample dilution with Dilution Buffer **DB** (e.g. 1:101).
N	Reconstitute with **100 µL A. dest**. Further dilution according to sample dilution with Dilution Buffer **DB** (e.g. 1:101).
O	Reconstitute with **1 mL** Assay Buffer **A.** Transfer dissolved material to Reagent **P** immediately before use. For 100 tubes add 1 vial reagent **O** (reconstituted in **1 mL A**) to 1 bottle of reagent **P** (**55 mL**) or any volumes in the same ratio (1:56) for less tubes. The assay is unaffected by the possible occurrence of turbidity in the final reagent.

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8 ASSAY PROCEDURE

Flow Chart of Assay Protocol:

Nr. of tubes	Contents of tube	DB E-L M,N Samples	D	B	C	P
1, 2	Total Counts	---	---	---	100	---
3, 4	NSB	100 **DB**	100	---	100	500
5, 6	B_0	100 **E**	---	100	100	500
7 - 20	Calibrators	100 **F-L**	---	100	100	500
21, 22	High Control	100 **M**	---	100	100	500
23, 24	Low Control	100 **N**	---	100	100	500
25, 26	Sample 1	100	---	100	100	500
27, 28	Sample 2	100	---	100	100	500
etc.						
Colour after addition:		Green	Blue		Violet	

Note: All volumes are given as µL.

Samples (calibrators, controls and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the test-protocol are recommended. Before use, all kit components should be brought to room temperature at **20 - 25 °C** (68 - 77 °F), except reagent **P**.

1) Labelling of the assay tubes (duplicates) should be done in the following order: 1 and 2 **total counts**, 3 and 4 **NSB**, 5 and 6 **zero calibrator** (**B_0**), 7 to 20 **calibrators**, 21 to 24 controls, 25 to 100 **samples**.
2) Add **100 µL** of Dilution Buffer **DB** to tubes 3 and 4.
3) Add **100 µL** of reagents **E - L (calibrators)** to tubes 5 to 20, (**zero calibrator (E)** to tubes 5 and 6, **calibrator F** (0.156 ng/mL) to tubes 7 and 8, etc).
4) Add **100 µL** of diluted reagent **M** (**high control**) to tubes 21 and 22 and **100 µL** of diluted reagent **N** (**low control**) to tubes 23 and 24.
5) Add **100 µL** of diluted (or only acidified) **samples** to tubes 25 and 26, etc. - All solutions appear **green**!
6) Add **100 µL** reagent **D (NSB)** to tubes 3 and 4. -The solutions turn **blue.**
7) Add **100 µL** reagent **B (1st Antibody)** beginning with tube 5. -The solutions turn **blue**!
8) Add **100 µL** reagent **C** (tracer) to all tubes. - All solutions turn **violet**!
9) Remove tubes 1 and 2 (**total counts**) or mark or seal with a stopper.
10) Mix tubes with a vortex mixer.
11) Incubate tubes at **2 - 8 °C (35.6 - 46.4 °F)** for **2 days**. Incubation for a longer period (e.g. **over the weekend**) has no negative effect on the results.
12) Add **500 µL** reagent **P** (after addition of reagent **O**), beginning with tube 3. The reagent should be cold **2 - 8 °C (35.6 - 46.4 °F).**
13) Mix tubes with a vortex mixer.
14) Incubate tubes at **2 - 8 °C (35.6 - 46.4 °F)** for **1 hour**.
15) Add **1 mL ice-cold distilled water**.
16) Centrifuge all tubes except tubes 1 and 2 at least at **3000 x g** for **30 min** at a temperature of **2 - 8 °C (35.6 - 46.4 °F).**
17) Aspirate the supernatant (except tubes 1 and 2 !). The remaining supernatant should be about 2 mm above the precipitate. Take care that the precipitate remains intact. Depending on local conditions and procedures, the supernatant may also be decanted instead of aspirated.
18) Count the activity of **all** tubes (including tubes 1 and 2) for **1** to **3 min**.

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9 CALCULATION OF RESULTS

9.1 Establishing of the Calibrator Curve

The calibrators provided contain the following concentrations of IGF-I:

Calibrator	E	F	G	H	I	J	K	L
ng/mL	0.0	0.156	0.313	0.625	1.25	2.5	5.0	10

1. Calculate the average counts of each pair of tubes.
2. Subtract the average counts of NSB tubes (3 and 4) from the mean counts of the calibrators, controls and samples. This gives the corrected values for B.
3. The corrected value from the zero calibrator E (tubes 5 and 6) is B_0.
4. Calculate the percent bound (% B/B_0) by dividing the corrected B-values by B_0: B/B_0 x 100%.
5. Plot % B/B_0 versus the calibrator concentrations on either semi-logarithmic or logit-log paper. For convenience, it is recommended to use computer assisted data reduction programs.
6. For quality control calculate NSB in %: average counts of tubes 3 and 4 divided by the average counts of tubes 1 and 2 (Total Count, TC) times 100%. It should be < 5% (%NSB/TC< 5).

   Calculate the percent bound of the zero calibrator E: average counts of tubes 5 and 6 minus average counts of NSB divided by TC times 100%. It should be > 25% (%B_0/TC > 25).

Example:	
unspecific Binding in [%:]	**specific** Binding in [%]:
NSB / Total activity TC x 100 = 510 / 23435 x 100 = 2.2%	B0 / Total activity TC x 100 = (10984 – 510) / 23435 x 100 = 44.7%

	Example	Target Value
Unspecific Binding**:%NSB/TC**	2.2	< 5.0
Specific Binding **%B0/TC**	44.7	> 25

9.2 Example of Typical Calibrator Curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

	E (B0)	F	G	H	I	J	K	L	TC	NSB
ng/mL	0.0	0.156	0.313	0.625	1.25	2.5	5.0	10	-	-
cpm	10984	10369	9689	8720	7270	5461	3709	2423	23435	510

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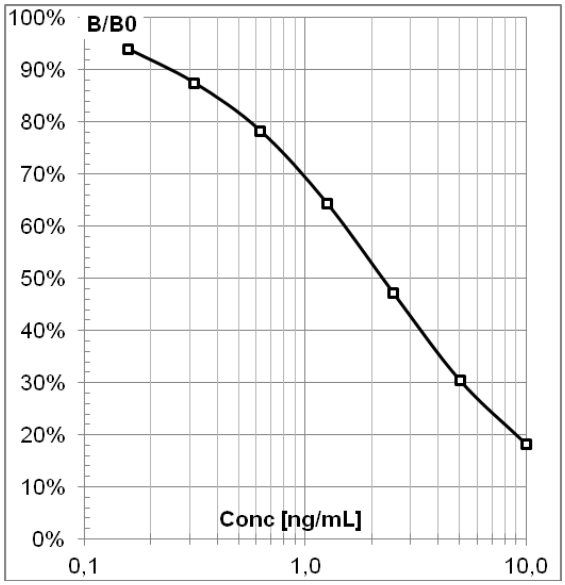

Figure 2 Exemplary Calibrator Curve.

9.3 Evaluation of sample concentration

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

Average counts of **NSB** (**D):**	**510 cpm**
Average counts of zero calibrator **E (B0):**	**10984 cpm**
Average counts of sample:	**6311 cpm**

%**B/B0** = (**sample counts**- **NSB**) / (**B0** - **NSB**) **x 100**
= (6311 - 510) / (10984 - 510) x 100
= 0.5539 x 100
= **55.39 %**

For a 55.39 %-value on the y-axis (ordinate) a value of 1.81 ng/mL on the x-axis (abscissa) was obtained. Multiply the concentration value determined graphically or by the aid of a computer program with the dilution factor (e.g.: 101).
Example: 1.81 x 101 = 183 ng/mL
In order to express the results as nmol/L the values given as ng/mL should be divided by 7.649 (molecular weight of IGF-I in kilo dalton).
Example: 183 ng/mL: 7.649 = 23.9 nmol/L

9.4 Concentration of control samples

The IGF-I concentration of Controls **M&N** should be within the ranges given on the certificate.

10 LIMITATIONS OF PROCEDURE

Generally, immunological assays are sensible to heterophilic antibodies and rheumatoid factors in the sample. Their influence is reduced by the assay design, but cannot be excluded completely.

11 EXEMPLARY VALUES

IGF-I levels are highly age-dependent in children, less so in adults until the age of about 60. The exemplary ranges in various age groups, which are log-normally distributed, are given in Table 2 by percentiles.
Between 8 and 19 years of age, values are given for boys and girls separately, because the pubertal peak usually occurs approximately 2 years earlier in girls.

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Table 1 Exemplary range of serum IGF-I levels given in ng/mL at different pubertal stages according to Tanner. Because no significant difference between boys and girls is observed, both sexes are combined. Only children and adolescents between 7 and 17 years of age are included.

Percentile				
Pubertal Stage	**0.1th**	**5th**	**50th**	**95th**
1	61	105	186	330
2	85	156	298	568
3	113	196	352	631
4	171	268	431	693
5	165	263	431	706

Table 2 Serum levels of IGF-I in healthy subjects at various ages. Individuals between 8 and 19 years of age were classified according to gender, as the pubertal peak occurs almost 2 years earlier in girls than in boys.

Percentile														
Age	**0.1**	1	**5**	10	**20**	30	**40**	50	**60**	70	**80**	90	**95**	99
0-2 y.	**13**	20	**28**	34	**43**	50	**58**	66	**75**	87	**102**	128	**156**	220
2-4 y.	**20**	29	**40**	48	**59**	68	**77**	87	**98**	111	**129**	159	**189**	260
4-6 y.	**26**	36	**50**	59	**73**	85	**96**	108	**122**	138	**160**	196	**233**	320
6-7 y.	**34**	46	**62**	72	**87**	99	**111**	124	**138**	155	**176**	212	**248**	332
7-8 y.	**45**	60	**78**	90	**107**	121	**134**	148	**163**	181	**205**	243	**281**	364
8-9 y. boys	**54**	71	**90**	102	**119**	133	**146**	160	**175**	192	**214**	250	**284**	362
girls	**55**	75	**99**	115	**137**	156	**174**	193	**214**	239	**271**	324	**376**	496
9-10 y. boys	**63**	82	**102**	115	**133**	148	**162**	176	**191**	209	**232**	269	**304**	379
girls	**68**	89	**114**	130	**152**	170	**187**	205	**224**	247	**276**	323	**369**	469
10-11 y. boys	**77**	96	**117**	130	**148**	162	**176**	189	**203**	220	**241**	274	**305**	370
girls	**81**	106	**134**	153	**178**	199	**219**	239	**261**	287	**321**	374	**426**	539
11-12 y. boys	**85**	106	**129**	144	**163**	179	**194**	209	**225**	244	**267**	304	**339**	413
girls	**91**	123	**160**	185	**220**	248	**276**	305	**337**	374	**424**	503	**581**	758
12-13 y. boys	**88**	112	**141**	159	**184**	204	**223**	243	**264**	289	**321**	371	**419**	525
girls	**116**	155	**201**	231	**274**	309	**342**	377	**415**	460	**519**	614	**707**	914
13-14 y. boys	**111**	143	**179**	203	**235**	261	**286**	311	**339**	371	**412**	477	**540**	677
girls	**163**	207	**256**	287	**329**	364	**395**	428	**463**	504	**556**	637	**716**	884
14-15 y. boys	**140**	182	**229**	260	**303**	337	**370**	404	**441**	484	**539**	625	**691**	896
girls	**193**	236	**284**	314	**353**	385	**414**	443	**474**	510	**556**	628	**713**	832
15-16 y. boys	**176**	221	**269**	299	**340**	372	**402**	433	**466**	504	**552**	626	**697**	849
girls	**187**	231	**279**	309	**350**	382	**412**	442	**474**	512	**559**	632	**700**	845
16-17 y. boys	**178**	221	**267**	296	**335**	366	**395**	424	**455**	491	**537**	607	**673**	814
girls	**183**	225	**270**	298	**336**	366	**394**	422	**452**	486	**530**	597	**660**	792
17-18 y. boys	**173**	207	**243**	265	**294**	317	**337**	358	**380**	405	**436**	484	**527**	618
girls	**176**	210	**246**	268	**297**	320	**341**	362	**384**	409	**441**	488	**533**	624
18-19 y. boys	**167**	201	**235**	256	**285**	307	**327**	347	**368**	393	**423**	469	**512**	600
girls	**167**	199	**233**	254	**281**	302	**322**	341	**362**	385	**414**	458	**499**	583
19-20 y.	**158**	189	**220**	240	**265**	285	**304**	322	**341**	363	**391**	433	**471**	550
20-30 y.	**72**	92	**115**	130	**150**	167	**182**	198	**215**	235	**261**	302	**340**	425
30-40 y.	**68**	87	**109**	123	**142**	158	**173**	188	**204**	223	**248**	287	**324**	404
40-50 y.	**64**	82	**103**	116	**135**	150	**164**	178	**194**	212	**235**	272	**310**	385
50-60 y.	**60**	77	**97**	110	**127**	142	**155**	169	**184**	201	**224**	260	**292**	369
60-70 y.	**55**	72	**91**	103	**120**	134	**147**	161	**176**	193	**215**	251	**282**	362
70-80 y.	**25**	35	**47**	55	**67**	78	**88**	98	**110**	124	**142**	173	**207**	276
>80 y.	**21**	30	**40**	47	**58**	67	**76**	85	**95**	108	**125**	153	**184**	245

Serum concentrations are given in ng/mL. Determined with IGFBP-blocked IGF-I RIA without extraction step (Blum and Breier 1994) (27).

Exemplary values have been evaluated by Prof. Blum by a radioimmunoassay identically composed to Mediagnost R20. Thus, these age and sex specific exemplary values can be applied to all Mediagnost IGF-I assays.

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12 PERFORMANCE CHARATERISTICS

12.1 Sensitivity

Sensitivity was assessed by measuring the B_0 in 16-fold determination and calculating the theoretical concentration of the CPM of B_0 (Mean Value - 2SD). The analytical sensitivity of the Mediagnost R20 is calculated to 0.064 µg/L (range: 0.02-0.109).

12.2 Specificity

The following materials have been evaluated for cross reactivity. 200 ng/mL solutions of each substance have been analysed in this Radioimmuimmunoassay. No significant cross reactivity of the tested substances was detected (see table 3).

Table 3 Cross reactivity of IGF-I related proteins

	IGF-II	Insulin	Proinsulin	C-Peptide
Reactivity [%]	0.103	0.005	0.012	0.019

12.3 Precision Data

Intra-Assay Variance

Six samples have been measured two to four times in the same assay. The results are shown in Table 4. The measured coefficient of variation (CV) is 4.76% on average (Range: 1.0 -16.1%).

Table 4 Intra-Assay Variation was measured in independent test with different lots. Each sample was measured twice or four times within each assay and the %CV was calculated for each sample and each test.

%CV	Test 1	Test 2	Test 3	Test 4
Sample 1	1.8	1.9	3.4	4
Sample 2	3.4	11.4	6.4	16.1
Sample 3	4.6	5.8	2.8	6.3
Sample 4	3.3	2.0	2.8	4.7
Sample 5	3.9	6.2	1.0	7.3
Sample 6	2.7	3.2	2.9	6.3
Mean [%]	3.28	5.08	3.22	7.45

Inter-Assay Variance

Serum samples where measured in independent assays. On average the coefficient of variation was 5.06% (Range 4.46 – 6.00%). Exemplary results are shown in table 5.

Table 5 Inter-Assay Variation measured as %CV of n-fold measured IGF-I concentration of different human serum in different kit lots within 18 months.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Mean [µg/L]	300	263	172	200	298	151	428	309	196
SD [µg/L]	13.40	12.82	8.62	9.54	15.81	9.09	22.05	16.12	9.32
%CV	**4.46**	**4.87**	**5.01**	**4.77**	**5.30**	**6.00**	**5.16**	**5.22**	**4.75**
n	62	49	43	60	58	62	62	53	58

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Lot-to-Lot Variability

Several samples have been tested several times in different lots. In the below table the results of five serum samples are summarized.

Table 6 Lot-to-Lot variability of IGF-I measurements. Exemplary results are shown for 5 serum samples measured repeatedly over a period of five years in 9 different lots.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	209.98	63.44	179.85	152.02	552.73
SD	9.98	3.98	11.84	9.26	38.90
CV%	4.76	6.28	6.58	6.09	7.04
n	159	159	160	120	78

SD=Standard Deviation, CV =coefficient of variation, n=Number

12.4 Linearity

Two samples were diluted, each in two independent assays, and IGF-I concentration was measured in each dilution. In table 7 the recalculated IGF-I concentrations are shown.

Table 7 Linearity: recalculated IGF-I concentrations of different diluted samples. The recommended dilution is 1:101.

Dilution:	Sample 1 (calculated, ng/ml)	Sample 1 (calculated, ng/ml)	Dilution:	Sample 2 (calculated, ng/ml)	Dilution:	Sample 2 (calculated, ng/ml)
1:50	480.2	521.8	1:30	161.1	1:25	136.0
1:100	496.6	525.1	1:60	169.8	1:50	142.0
1:200	520.6	507.4	1:120	170.8	1:100	141.7
1:400	531.2	520.0	1:240	174.5	1:200	141.4
1:800	572.0	570.4	1:480	174.7	1:400	134.0
1:1600	604.8	563.2	-	-	-	-

12.5 Recovery

Recombinant IGF-I was added in different amounts to human serum. The IGF-I content of the so enriched samples was measured. Results are shown in table 8.

Table 8 Recovery of recombinant human IGF-I in serum.

	IGF-I [µg/L]		% recovery	
added IGF-I [µg/L]	100	400		
Sample 1	190	538	106	112
Sample 2	283	623	101	107
Sample 3	265	592	110	110

12.6 Trueness / Assay Calibration

Recombinant IGF-I produced by E. coli and of >98% purity (SDS-PAGE, Silverstain) is used as calibrator within the assay. The traceability of this recombinant calibration material to the international reference material of the WHO 02/254 has been proven. Results are published by Burns C et al. in Growth Horm IGF Res. 2009 Oct; 19(5):457-62. Epub 2009 Mar 20. Mediagnost R20 is coded by 14a.

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The reference material includes 8.5 µg/ampoule IGF-I measured by amino acid analysis and HPLC. Mediagnost R20 IGF-I immunoassay (14a) measures 12.87 µg/ampoule. The mean of all tested immunoassays is 11.61 µg/ampoule.
Thus, Mediagnost results are comparable to other immunological tests for measurement of IGF-I and can easily be transformed to WHO 02/254 by a division with: 1.514.

12.7 Cross reactions with animal samples

Several commercially available animal sera have been used as samples in this assay and therewith it is proven, that the test can be used as heterologous assay for IGF-I measurement in serum samples of primates, rats, mice, cattle, pig, sheep, horse, donkey, goat, dog, cat and guinea pig. Species specific calibration has to be done by the user.

13 ASSAY COMPARISON

Mediagnost R20 was compared with two other commercial available assays. Evaluation was conducted by an independent third party and results were published by a peer-reviewed journal (28).

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15 ASSAY PROCEDURE

Reagent preparation:		Reconstitution:	Dilution:
B	**1. Antibody**	in **11 mL** Assay Buffer **A**	**-**
C	**Tracer**	in **11 mL** Assay Buffer **A**	**-**
D	**NSB**	in **500 µL** Assay Buffer **A**	**-**
E-L	**Calibrators**	in **500 µL** Dilution Buffer **DB**	**-**
M+N	**Controls**	in **100 µL Aqua dest.**	**1:101** with **DB**
O	**2. Antibody**	in **1 mL** Assay Buffer **A** **Mix** immediately before use with **55 mL** Reagent **P (1:56)**	
Dilute **Samples** with Dilution Buffer **DB 1:101.**			
Before use, all kit components should be brought to room temperature at **20 - 25 °C** (68 - 77 °F), except reagent **P**.			

Assay procedure for double determination

Nr. of Tubes	Contents of Tubes	DB, E-L M, N Samples	D (NSB)	B (1.Antibody)	C (Tracer)
1 / 2	Total Counts	–	–	–	100 µL
3 / 4	NSB	100 µL **DB**	100 µL	–	100 µL
5 / 6	B0	100 µL **E**	–	100 µL	100 µL
7 - 20	Calibrators	100 µL **F-L**	–	100 µL	100 µL
21 / 22	High Control	100 µL **M**	–	100 µL	100 µL
23 / 24	Low Control	100 µL **N**	–	100 µL	100 µL
25 / 26	Sample 1	100 µL	–	100 µL	100 µL
27 / 28	Sample 2	100 µL	–	100 µL	100 µL
etc.					
Colour after addition		Green	Blue		Violet

Nr.: 1, 2 remove until counting the activity.
Mix other tubes with a Vortex-Mixer.
Incubation at 2 - 8 °C (35.6 - 46.4 °F), at least 40 hours (max. 92 hours)
Add **500 µL P** (after addition of reagent **O**) **in all Tubes** The reagent-mix should be cold **2 - 8 °C (35.6 - 46.4 °F)**
Mix with Vortex-Mixer.
Incubation at 2 - 8 °C (35.6 - 46.4 °F), 1 h
Add 1 mL ice-cold A. dest.
Centrifugation at ≥ **3000 x g, 30 min**, **2 - 8 °C (35.6 - 46.4 °F)**
Aspirate the supernatant (as a precaution, e.g. leave approx. 2 mm as a remaining supernatant above the precipitate).
Count the **activity** of all tubes with a Gamma Counter.

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