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IGF-I RIA-CT

Radioimmunoassay with Coated Tubes for Quantitative Determination of

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

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

+2°C +8°C

100 Tubes

REF **R22**

mediagnost®
Gesellschaft für Forschung und Herstellung von Diagnostika GmbH

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

IGF-I RIA-CT, R22

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Instructions for Use

IGF-I RIA-CT, R22	100 Determinations
Principle of the test	Radioimmunoassay
Duration (incubation period)	4 h
Tracer	Iodinated recombinant human IGF-I, < 55 kBq
Antibodies	specific, high-affinity polyclonal antiserum
Cross reactivity with IGF-II; Insulin; Proinsulin	< 0.1%
Buffer	Ready to use
Calibrator	5 single calibrators: 0.37 -30 µg/L, Recombinant human IGF-I, ready to use
Assay Range	0.25 – 780 µg/L
Control	2 control sera, freeze-dried - RiliBäK conform
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:26
Analytical sensitivity	< 0.25 µg/L
Intra- / Interassay Variance	ø <10 %
Half Maximal displacement	at < 5 µg/L

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.

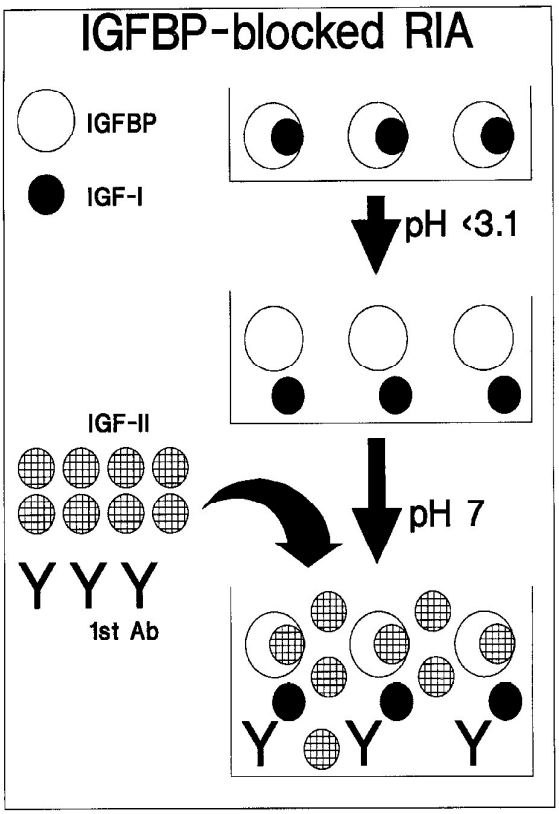
-in samples with high IGF-I concentration, unmarked IGF-I from the sample will be predominantly complexed by IGFBPs and therefore withdrawn from measurement. Effect: Underestimation of high IGF-I levels.

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 streptavidin-coated 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.



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. In order to separate bound and free tracer, the immuno-complex (antigen-spec. antibody) binds to the capture antibody that binds in turn to the streptavidin-coated tubes. Therefore, time-consuming centrifugations and separations become unnecessary.
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 Buffer (calibrators and diluted samples too) are coloured in green by 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.

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

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.

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.

5.2 Specimen collections

Use calibrator 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: 26** with Dilution Buffer **DB**
- **Example: 10 µL** sample to **250 µl** Dilution Buffer **DB** provided (26 dilution factor).

For samples as expected with extremely low IGF-I contents, non-serum or plasma samples (e.g. saliva or urine) the more sensitive Mediagnost R20 might be better suited.
Attention: serum and plasma samples must be diluted at least 1:20 in Dilution Buffer DB in order to achieve sufficient acidification of the samples.

5.7 Warning Statement

Biotin concentrations of >375 ng/mL in R22 showed significant interference with the assay system and result in false high values. Therefore samples from patients under high dose biotin therapy (>10 mg/day) cannot be used for IGF-I measurement by this test.

6 MATERIALS PROVIDED

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

DB	**Dilution Buffer**, ready to use, coloured	**1 x 125 mL**
A	**Assay Buffer** ready to use	**1x 30 mL**
A	**Assay Buffer** ready to use	**1x 60 mL**
R	**Capture Antibody,** lyophilized, anti-rabbit-IgG, biotin-conjugated	**1 x 5.5 mL**
S	**Specific Antibody,** lyophilized, rabbit-anti-hIGF-I (containing recomb. hIGF-II)	**1 x 5.5 mL**
C	**Tracer: ^{125}I-IGF-I,** lyophilized, < 1.5 µCi or < 55 kBq - red coloured	**1 x 11 mL**
F-J	**Calibrators,** ready to use (rec. Human IGF-I) Concentrations given on vial-labels	**5 x 750 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**
T	**Tubes** (100 tubes, coated with streptavidin)	**100 tubes**
	**Instructions for use**	**1 x**
	Quality Certificate	**1 x**

6.1 Materials required, but not provided

- Demineralised water or distilled water (Aqua destillata) (A. dest.)
- Pipettes: 10 mL, 1 mL, 250 µL, 100 µL, 10 µL,
  100 µL, 250 µL, 500 µL repeating pipettes are recommended.
- Shaking device
- Vortex mixer
- Device for aspiration of liquid supernatant
- Gamma counter

7 REAGENT PREPARATION

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.**

Possible residues on the coated tubes **T** are unavoidable for production reasons - the function is not impaired.

C	Reconstitute with **11 mL** Assay Buffer **A**
R	Reconstitute with **5.5 mL** Assay Buffer **A**
S	Reconstitute with **5.5 mL** Assay Buffer **A**
M and N	Reconstitute with **100 µL** Aqua dest., each. Ensure that lyophilized materials are completely dissolved on reconstitution. Further dilution according to sample dilution with the dilution buffer DB (e.g. 1:26)

8 ASSAY PROCEDURE

Flow Chart of Assay Protocol:

Nr.	Tubes	DB F-N Samples	S	R	C
1,2	TC	---	---	---	100
3,4	B_0	**DB**: 100	50	50	100
5-14	Calibrators	**F-J**: 100	50	50	100
15,16	High Control	**M**: 100	50	50	100
17,18	Low Control	**N**: 100	50	50	100
19,20	Sample 1	100	50	50	100
21,22	Sample 2	100	50	50	100
etc.					
Colour :		**Green**	After addition: **Blue**		After addition: **Violet**

Note: All volumes are given as µL.

Samples (calibrators and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the test-protocol are recommended.

1) Labelling of the assay tubes should be done in the following order:

1, 2	total counts (**TC**)
3, 4	**Dilution Buffer DB** (zero calibrator, $\mathbf{B_0}$)
5 - 14	duplicates of **Calibrators (F to J)**,
15, 16	duplicates of **High Control (M)**,
17, 18	duplicates of **Low Control (N)**,
19, 20 etc.	duplicates of **samples**

2) Add **100 µL** of **Dilution Buffer DB** to tubes 3 - 4.

3) Add **100 µL** of reagents **F - J (Calibrators)** to tubes 5 - 14:

5, 6	Calibrator F
7, 8	Calibrator G, etc.

4) Add **100 µL** of **diluted reagent M (High Control)** to tubes 15 and 16.

5) Add **100 µL** of **diluted reagent N (Low Control)** to tubes 17 and 18.

6) Add **100 µL** of diluted sample to tubes 19 and 20, etc.

-All solutions appear **green**!-

7) Add **50 µL** reagent **S (Spec. Antibody)** beginning with tube 3.

-All solutions turn **blue**!-

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

9) Add **100 µL** reagent **C (tracer)** to all tubes.

-All solutions turn **violet**!-

Remove tubes 1 and 2 (total counts, **TC**) or seal with a stopper.

10) **Shake** the tubes on a shaking device **(350 rpm) at least 4 h** at **room temperature**.

Alternative Incubation:

A) Over night: (>15 h), 350 rpm, RT, 20 - 25 °C (68 - 77 °F)

B) Over night: (>15 h), without shaking, 2 - 8 °C (35.6 - 46.4 °F)
(In this case contents of the tubes must be initially mixed by unique shaking or vortexing)

C) Extended Incubation: For example over the weekend (e.g. 60 h) is possible with the version B

11) Aspirate the liquid (except tubes 1 and 2!) completely.

Take care that the coating of the tubes remains intact. Depending on laboratory equipment and common laboratory practice, aspiration of the liquid can be replaced by careful decantation.

12) Add **500 µL** of reagent **A (Assay Buffer)** to the tubes
(except tubes 1 and 2!).

13) Aspirate the liquid (see step 11).

14) Count the radioactivity of **all** tubes.

Alternative Pipetting Schema for working steps 7 and 8

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.

9 ESTABLISHING OF THE CALIBRATION CURVE

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

Calibrator	F	G	H	I	J
ng/mL	0.37	1.11	3.33	10	30

1. Calculate the average counts of each pair of tubes. This equals to the binding value B.
2. The average value from the zero calibrator (tubes 3 and 4) is B_0.
3. Calculate the percent bound (% B/B_0) by dividing the average B-values by B_0: B/B_0 x 100%.
4. 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.
5. Calculate the 'percentage bound of the zero calibrator':
   B_0 / total counts TC x 100%
   = (average of tubes 3, 4 / average of tubes 1, 2) x 100%.
   It should be $\%B_0/TC > 25\%$.

9.1 Example of Typical Calibration Curve

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

	(B0)	F	G	H	I	J	TC
ng/mL	**0.0**	**0.37**	**1.11**	**3.33**	**10**	**30**	
cpm	7856.8	7404.7	6299.8	4363.1	2296.3	970.6	17712.6

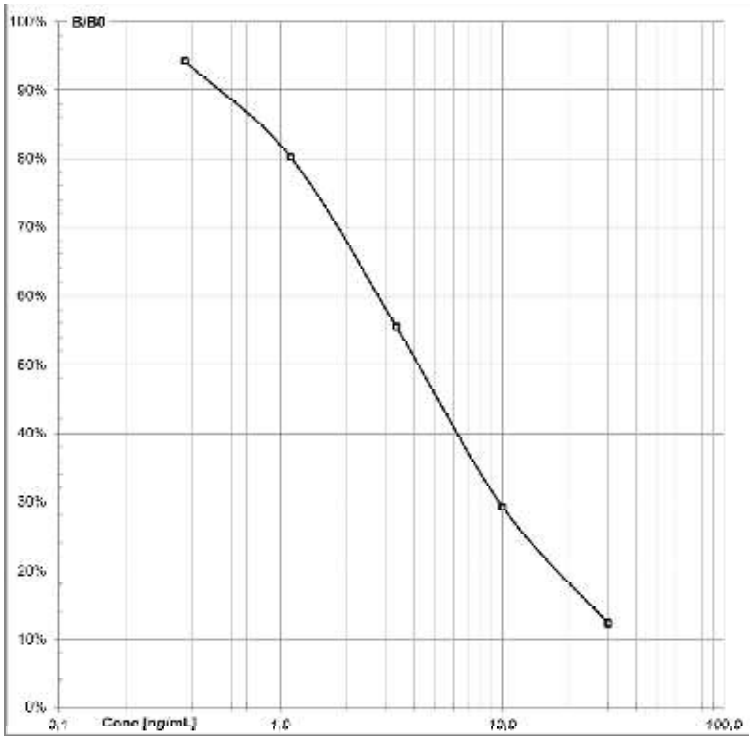


Figure 2: Exemplary Calibration Curve

9.2 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 zero calibrator (B0): 7856.8 cpm
average counts of sample: 2961.9 cpm
%B/B0 = (sample-counts) / (B0) x 100%
= 2961.9 / 7856.8 x 100%
= 0.377 x 100%
= 37.7%

For a 37.7% value on the y-axis (ordinate) a value of 6.79 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.
Example: 6.79 x 26 = 177 ng/mL.
If it is preferred to express the results as nmol/L, the values given as ng/mL should be divided by 7.649 to obtain nmol/L.
Example: 177 ng/mL : 7.649 = 23.1 nmol/L

9.3 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.

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.

12 PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

Analytical sensitivity was determined by measuring the zero calibrator B_0 16-fold in the assay and calculating the theoretical concentration from mean B_0 minus 2 standard deviations.
The analytical sensitivity of Mediagnost R22 is less than 0.25 ng/mL (< 0.25 ng/mL, mean 0.087 ng/mL, range: 0.015 – 0.224 ng/mL, n = 54 in the 10-year period 2012 - 2021).

12.2 Specificity

Measurement of R22 cross reactivity with IGF-II, Insulin, Proinsulin and C-Peptide. These IGF-related proteins were added to assay buffer in the indicated concentration and the solution was applied as sample without any further dilution. No significant cross reactivity of the tested substances was detected (see table 3).

Table 3 Specificity Cross-reactivity with homologous proteins. Recombinant proteins were diluted in Dilution Buffer **DB** to 200 ng/mL and treated as a sample. The signal intensity was measured in the Mediagnost IGF-I RIA, R22.

	measured IGF-I [µg/L]	Cross -reactivity [%]
Insulin	0.121	0.061
Proinsulin	0.047	0.024
C-peptide	0	n.d
IGF-II	0.042	0.021

12.3 Precision

Intra-Assay Variance

Three samples have been measured 8 times in the same assay. The results are shown in table 4. The measured coefficient of variation (CV) is 4.29% on average. Further, 10 samples were measured four-fold and the resulting mean %CV was 4.1.

Table 4 Intra-Assay Variation

	Sample 1	Sample 2	Sample 3
	183.6	56.4	140.5
	186.5	58.4	132.4
	173.1	63.7	136.2
	186.1	53	139.5
	173.2	54.1	135.3
	186.2	54.6	146.8
	170.2	51.6	150.8
	177.8	54.1	143.2
Mean	180	56	141
SD	6.37	3.57	5.76
%CV	3.54	6.41	4.09

Inter-Assay Variance

Serum samples were measured in independent assays. On average the coefficient of variation was 5.88%. Results are shown in detail in table 5.

Table 5 Inter-Assay Variation. Five samples have been tested during 4 years numerous times in several lots. The mean coefficient of variation was 5.88%.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	203	60	173	128	91
SD	16	4	10	6	4
%CV	7.94	5.90	5.83	5.02	4.68
n	108	108	104	67	59

12.4 Linearity

Linearity of the R22 was tested by dilution of different serum samples and recalculation of the measured IGF-I concentration. Exemplary results are shown in figure 3. Samples can be diluted in broad range (1:20 – 1:832) according the requirements of the experimental setting. We recommend a sample dilution of 1:26.

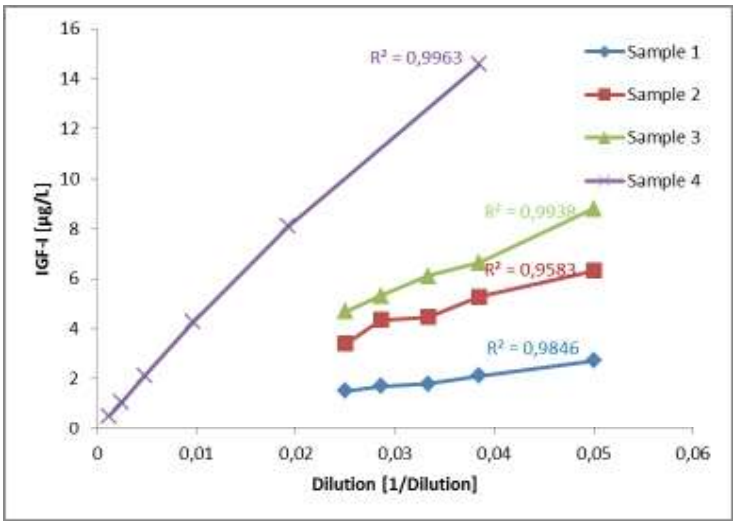


Figure 3 Linearity of sample dilution. Four samples with different amount of IGF-I were diluted (1:20 – 1:832) and IGF-I concentration was recalculated.

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 and recovery calculated in comparison to endogenous + added IGF-I. Results are shown in table 6.

Table 6 Recovery of recombinant human IGF-I in serum

	IGF-I [µg/L]	% recovery
Sample 1	222	105
Sample 2	171	104
Sample 3	264	90
Sample 4	235	90

12.6 Interference

Assessment of the influence of hemolytic, icteric and lipaemic samples was done by an artificial system. Exemplary three 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 IGF-I was measured in the enriched and not enriched samples. Further, three different concentrations IGF-I were incubated with up to 12 µg/mL IGFBP-3 and IGF-I recovery was measured. No significant influence of the above mentioned concentrations of potentially interfering substances was detected (Table 7). But this might depend on the individual sample and thus hemolytic, icteric and lipaemic samples should be avoided. Also biotin interference was evaluated by enriching serum samples with biotin in different concentrations. Concentrations >375 ng/mL did interfere significantly with the IGF-I measurement.

Table 7 IGF-I measurements in artificially enriched samples

	Triglyceride 100 mg/mL	Bilirubin 200 µg/mL	Hemoglobin 5 mg/mL	IGFBP-3 12 µg/mL	Biotin 750 ng/mL
Serum 1	92	84	108	88	113
Serum 2	93	97	112	74	100
Serum 3	106	104	98	84	109

12.7 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 R22 IGF-I RIA-CT is coded by 14b.
The reference material includes 8.5 µg/ampoule IGF-I measured by amino acid analysis and HPLC. Mediagnost R22 IGF-I RIA-CT immunoassay (14b) measures 11.55 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: 1.366.

12.8 Calibration Curve

Uncertainty of the calibrators was measured as precision of recalculated calibrator concentration. Relative deviation corresponds to uncertainty. Results are shown in table 8.

Table 8 Uncertainty of calibrators R22.
Shown is the mean of the variance of recalculated calibrator concentration of 11 independent assays.

CAL	IGF-I [µg/L]	SD	%CV	n
F	0.37	0.023	6.10	11
G	1.11	0.034	3.13	11
H	3.33	0.028	0.83	11
I	10	0.069	0.69	11
J	30	0.099	0.33	11

12.9 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, cattle, pig, sheep, horse, donkey, goat, dog, cat, rabbit and guinea pig. For the determination of IGF-I in chicken, rat and mouse sera is this kit not usable.

Species specific calibration has to be done by the user.

To determine exact species-specific IGF-I concentrations, the test system must be calibrated respectively: for example, using bovine IGF-I in a known concentration.

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14 ASSAY PROCEDURE

Preparation of reagents		Reconstitution	Dilution
R	**Capture Antibody**, lyophilised	in **5.5 mL** Assay Buffer **A**	-
S	**Specific Antibody**, lyophilised	in **5.5 mL** Assay Buffer **A**	-
C	**Tracer,** lyophilised	in **11 mL** Assay Buffer **A**	-
F-J	**Calibrators,** ready to use	-	-
M	**Control Serum 1,** lyophilised	in **100 µL** A.dest. each	**1:26** with Dilution Buffer **DB**
N	**Control Serum 2,** lyophilised	in **100 µL** A.dest. each	**1:26** with Dilution Buffer **DB**
Dilute **Plasma/Serum Samples** with Dilution Buffer **DB** e.g. **1:26**.			

Before assay procedure bring **all** reagents to room temperature **20 - 25 °C** (68 - 77 °F).

Assay procedure in double determination

Addition of Reagent [µl]

Nr. of Tubes	Contents of Tubes	DB F-N Samples	S	R	C
1,2	Total Counts (**TC**)	–	–	–	100
3,4	B_0	100 **DB**	50	50	100
5-14	Calibrators	100 **F-J**	50	50	100
15,16	High Control **M**	100 **M**	50	50	100
17,18	Low Control **N**	100 **N**	50	50	100
19,20	Sample 1	100	50	50	100
21,22 (etc)	Sample 2 (etc)	100	50	50	100
Colour:		**green**	After addition: **blue**		After addition: **violet**

Tubes Nr.:1,2 remove until counting the activity

Incubation, at least. **4 hours**, at **RT, 20 - 25 °C** (68 - 77 °F)**, 350 rpm**

(Alternatively: Incubate **over night** without shaking (i.e., at least **15 hours**) at **2 - 8 °C** (35.6 - 46.4 °F)**,** mix tubes before incubation see page 10)

Aspirate the liquid completely. Take care that the coating of the tubes remains intact.

Add **500 µl** of Assay Buffer **A** to the tubes

Aspirate the liquid completely (see above)

Count the activity of all tubes with a Gamma Counter.