

IGF-I RIA-CT

**Radioimmunoassay with Coated Tubes
for the Quantitative Determination of**

Insulin-like Growth Factor-I

(IGFBP blocked)

Product Code: IGF-R22

100 tubes



DE/CA40/00809/5

European Union *

United States of America **

for in-vitro diagnostics

IVD for professional use!

Rest of the world: For research use only!



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TABLE OF CONTENTS

FEATURES	3
BACKGROUND	3
CLINICAL SIGNIFIGANCE	5
ASSAY PRINCIPLE	7
INTENDED USE	7
PRECAUTIONS	8
General	8
Radioactivity	10
Assay Characteristics and Validation	11
Clinical Validation	11
SAMPLE PREPARATION AND STORAGE	11
MATERIALS	12
Materials provided	12
Required Materials Not Provided	12
ASSAY PROCEDURE	13
Reagent Preparation	13
Sample Preparation	13
Procedure	14
EVALUATION OF RESULTS	17
Establishing of the Standard Curve	17
Evaluation of sample concentrations	17
EXPECTED VALUES	18
LIMITATIONS	19
REFERENCES	24
SUMMARY OF THE ASSAY	28

* Europäische Union / European Union: please ask for package inserts in your national language

** United States of America please ask for special package insert

Read entire protocol before use!

FEATURES

- ◆ High Specificity for IGF-I
- ◆ Correct measurement of IGF-I in non-extracted samples
- ◆ Elimination of interference by IGF-binding proteins through excess IGF-II
- ◆ No physical separation of IGF-I from IGF-binding proteins required
- ◆ Easy handling:
 - Standards ready for use
 - Separation without centrifugation by means of specifically coated tubes
 - More reliable performance by means of coloured solutions
- ◆ 100% recovery of IGF-I leads to correct absolute values
- ◆ Small sample volume requirement, thus ideal for young patients

BACKGROUND

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 (see also Figure 1):

- 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.

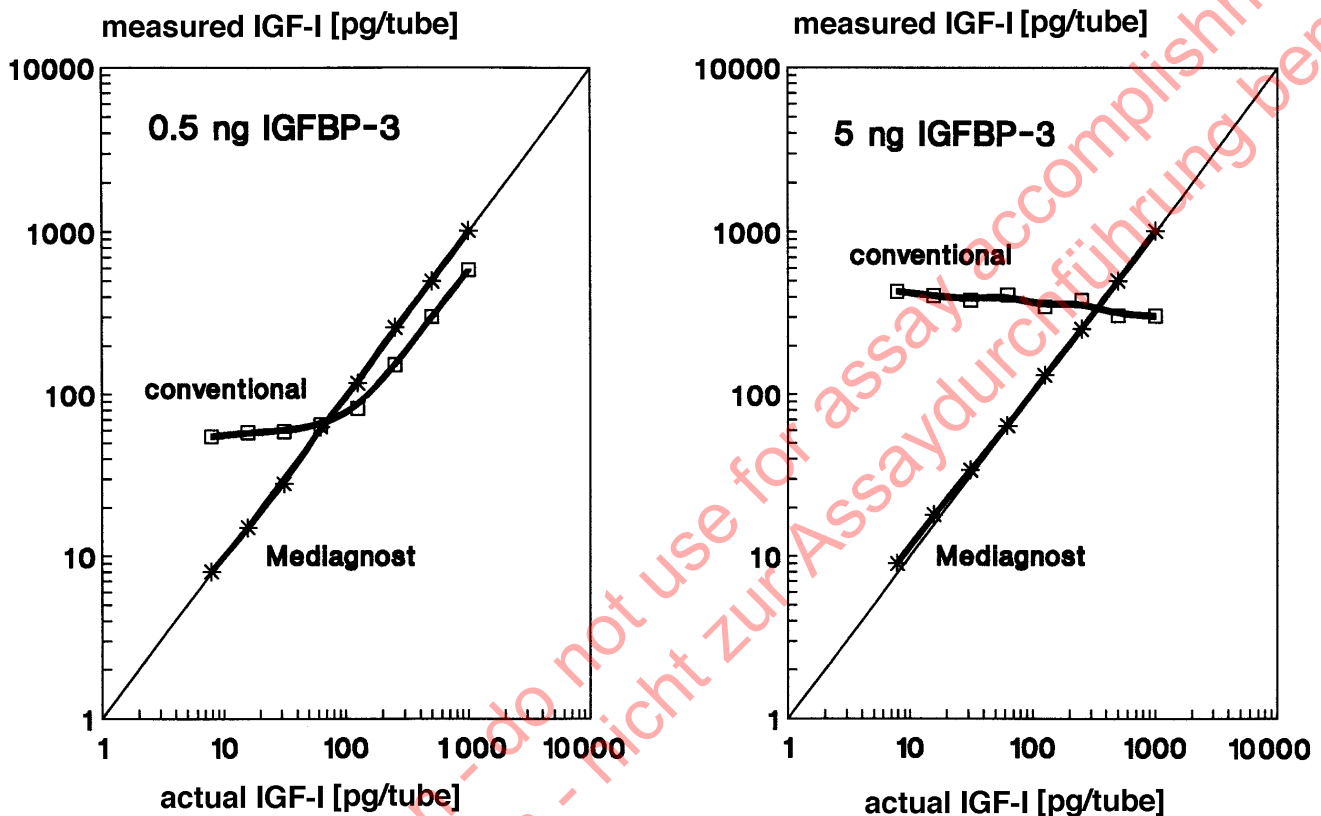


Figure 1. Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□) and by the IGFBP-blocked RIA (*).

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 and 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 (except dilution and/or acidification in a specially-composed buffer system).

CLINICAL SIGNIFICANCE

There are apart from GH, a number of variables that influence serum IGF-I. Decreased levels are found in states of malnutrition/malabsorption, hypothyroidism, liver disease, untreated diabetes mellitus, chronic inflammatory disease (1,6), malignant disease or polytrauma. High levels, on the other hand, are likely to be present in precocious puberty or obesity.

Crucially important to the correct interpretation of IGF-I measurements is the relationship between age and IGF-I levels. It is certainly inadequate to use a common cut-off point to define "normal" levels for all age groups, particularly in children and adolescents (for age-dependant serum levels see table 2 and figures 3-6).

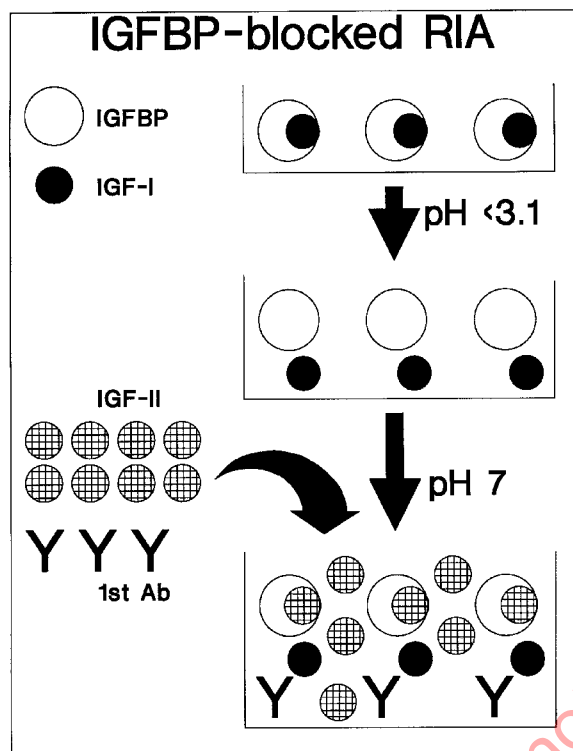
Due to its GH-dependence, determination of serum IGF-I was shown to be a useful tool in diagnosis of growth disorders, especially with regard to GH deficiency (GHD) or acromegaly (6,16-19,23,24). The major

advantage of IGF-I determination compared to GH determination is its stable circadian concentration, therefore a single measurement is sufficient. Hence IGF-I determination should be the first in a series of laboratory test. Clearly normal levels would then rule out disturbances of the GH-IGF-I-axis. Low levels, i.e. close to or below the age-related 5th percentile would indicate the necessity of further diagnostic efforts. Sub-normal levels of IGF-I would be evidence for reduced GH secretion, if other causes of low serum IGF-I (e.g. malnutrition or impaired liver function) can be ruled out. For differentiation of healthy short children without GH deficiency and children with "classical" GH deficiency, the 0.1st percentile proved to be an appropriate cut-off point, especially after the age of eight. However, IGF-I levels of short children not suffering from GHD may nevertheless lie between the 0.1st and 5th percentile (19). In contrast, acromegaly is characterized by pathologically elevated IGF-I levels which apparently reflect the severity of the disease better than GH-levels (17,18,20).

Exemplary version - do not use for assay development!
Exemplarische Version - nicht zur Assayentwicklung benutzen!

ASSAY PRINCIPLE

In order to dissociate IGF-I from the IGFBPs, the samples must be diluted in an acidic buffer (Figure 2). 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.

Figure 2 Principle of the IGFBP-blocked IGF-I RIA.

In order to separate bound and free tracer, the immuno-complex (anti-gen-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 pipetting plan, if necessary. Standards, dilution and acidification buffer (and by that, the 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 entire incubation colour **violet**.

INTENDED USE

This radioimmunoassay kit is suitable for the scientific and diagnostic measurement of IGF-I in human serum or plasma. Due to the high cross-reactivity of the specific antibody with IGF-I from other mammalian

species, it can also be used as a heterologous assay for determination of IGF-I in primates, cattle, pig, sheep, rat, mouse, cat, chicken, guinea pig, goat, horse, donkey and dog.

PRECAUTIONS

General

All reagents are for in vitro use only!

In conducting the assay, follow strictly the test protocol. The acquisition, possession and use of the kit is subject to the regulations of the national nuclear regulatory authorities.

Reagents with different lot numbers should not be mixed.

Reagents contain Sodium-Azide as preservative, however, highly diluted (0.02%). Safety Data Sheet available on request.

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 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 patient's specimens should be treated as potentially infectious.

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.

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 behaviour 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 $T_{1/2}$ of 60 days and emits 35,5 keV gamma radiation, 27 – 32 keV x-rays and no beta radiation. Shielding is effectively 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 air born, 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.

Warning Statement

Biotin concentrations of >375 ng/mL in IGF-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.

ASSAY CHARACTERISTICS AND VALIDATION

The radioimmunoassay for IGF-I uses a specific, high-affinity polyclonal antibody. Its cross-reactivity with IGF-II is less than 0.05 %. The sensitivity of the assay is approx. 0.1 ng/ml. The tracer is prepared through radioiodination of recombinant hIGF-I. The standards are derived from recombinant hIGF-I devoid of methIGF-I or IGF-I variants with mismatched disulfide bonds, i.e. this recombinant IGF-I is identical to the major authentic IGF-I form in blood.

Half-maximal displacement occurs at approx. 3 ng/ml. The inter-assay variation coefficient at 50 % B/B₀ is 7.4 .

The high sensitivity of the assay allows the measurement of IGF-I in small sample volumes which is limited by pipetting accuracy rather than the amount of IGF-I. Serum or plasma samples must be considerably diluted before measurement. No extraction step is required as in conventional IGF-I assays.

This radioimmunoassays is calibrated against the WHO International Reference Standard preparation of IGF-I, **WHO NIBSC 02/254** (25-26).

Clinical Validation

Clinical validation was achieved by determining the IGF-I levels in a large number of normal children and adults, normal short statured children without GH deficiency, girls with Ullrich-Turner syndrome, children with Silver-Russell syndrome, patients with GH deficiency, children with familial tall stature, Sotos syndrome, patients with acromegaly, and children with precocious puberty.

SAMPLE PREPARATION AND STORAGE

The stability of IGFBP-bound IGF-I makes sample preparation simple. Serum and Heparin/EDTA plasma levels are comparable. Citrated Plasma levels are reduced, because the anticoagulant amount dilutes the small sample slightly. 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 until measurement. IGF-I levels are usually not affected by improper handling or storage. They remain stable over several days in normal and in various clinical situations even under conditions of high temperature (37°C). Avoid repeated freezing and thawing cycles, although IGF-levels in normal sera remained unchanged

after 10 cycles. Frozen samples are stable over many years. Samples may also be freeze-dried without suffering any loss of activity.

Sample requirements: 10 µl serum or plasma.

Warning Statement

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

MATERIALS

Materials provided

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

- DB** Dilution Buffer
(1 bottle, 125 ml, ready for use, coloured)
- A** Assay Buffer
(2 bottles, 1 x 60 ml + 1 x 30 ml, ready for use)
- R** Capture Antibody: anti-rabbit-IgG, biotin-conjugated
(1 bottle, 5.5 ml, lyophilized)
- S** Specific Antibody: rabbit-anti-hIGF-I (containing recomb. hIGF-II),
(1 bottle, 5.5 ml, lyophilized)
- C** Tracer (125I-IGF-I) (< 1.5 µCi or < 55 kBq)
(1 bottle, 11 ml, lyophilized, red coloured)
- F -J** Standards. (Concentrations given on vial-labels in ng/ml)
(5 vials, 750 µl each, ready for use)
- M+N** Controls (Concentrations given on certificate in ng/ml)
(2 vials, 100 µl each, human serum, lyophilized)
- T** Tubes
(100 tubes, coated with streptavidin)

Required Materials Not Provided

- 1) Pipettes: 10 ml, 500 µl, 250 µl, 100 µl, 10 µl, 25 µl, 100 µl, and 250 µl repeating pipettes are recommended.
- 2) Shaking device
- 3) Device for aspiration of liquids (e.g. connected to a water pump).
- 4) Gamma counter

ASSAY PROCEDURE

Reagent Preparation

Store the kit at **2-8°C** after receipt until its expiry date. Reconstituted reagents should be stored at **-20°C**. Avoid repeated thawing and freezing cycles. The shelf-life of the reagents after opening is in accordance with the **Tracer C** shelf life.

R Reconstitute with **5.5 ml** reagent **A (Assay Buffer)**.

S Reconstitute with **5.5 ml** reagent **A (Assay Buffer)**.

C Reconstitute with **11 ml** reagent **A (Assay Buffer)**.

M+N Reconstitute with **100 µl** distilled water each.

Further dilution according to sample dilution with reagent **DB (Dilution Buffer)** (e.g. 1:26)

Ensure that lyophilized materials are completely dissolved on reconstitution. It is recommended to keep reconstituted reagents at room temperature 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!**

Sample Preparation

Normally serum or plasma samples have to be diluted with **Dilution Buffer DB**. This dilution with **Dilution Buffer DB** simultaneously leads to the necessary acidification to $\text{pH} \leq 3$ of the sample. For routine applications we recommend a dilution of 1:26.

Example: Add 10 µl sample to 250 µl **Dilution Buffer DB** (dilution factor 26).

The dilution of the **controls M & N** with **Dilution Buffer DB** should be according to the common dilution of serum or plasma samples, e.g. 1:26.

For samples as expected **with extremely low IGF-I contents**, non serum or plasma samples (e.g. Saliva or urine) the more sensitive **Mediagnost RIA IGF-R20** might be better suited.

Procedure

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

Exemplary version - do not use for assay accomplishment!
Exemplarische Version - nicht zur Assaydurchführung benutzen!

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	Standards	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

(All volumes are given as µl.)

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

- 1, 2 total counts (TC)
- 3, 4 **Dilution Buffer DB** (zero standard, **B₀**)
- 5 - 14 duplicates of **Standards (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 (Standards)** to tubes 5 - 14:

- 5, 6 Standard F
- 7, 8 Standard 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) Overnight: (>15h), 350 rpm, RT

B) Overnight: (>15h), without shaking, 2-8°C

(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.

EVALUATION OF RESULTS

Establishing of the Standard Curve

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

Standard	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 mean value of the Dilution Buffer (DB: tubes 3 and 4) equals B_0 .
3. Calculate the percent bound (% B/ B_0):
 $\%B/B_0 = B/B_0 \times 100\%$.
4. Plot % B/ B_0 versus the standard concentrations on a semi-logarithmic or logit-log paper respectively. For convenience, it is recommended to use computer assisted data reduction programs.
5. Calculate the 'percentage bound of the zero standard':
 $B_0 / \text{total counts TC} \times 100\%$
 $= (\text{average of tubes 3, 4} / \text{average of tubes 1, 2}) \times 100\%$
It should be $\%B_0/TC > 25\%$.

Evaluation of sample concentrations

Read the concentration value (abscissa) corresponding to the % B/ B_0 of the sample as in the example given below:

average counts of zero standard (B_0): 9005 cpm

average counts of sample: 3812 cpm

$$\%B/B_0 = (\text{sample-counts}) / (B_0) \times 100\%$$

$$= (3812) / (9005) \times 100\%$$

$$= 0.423 \times 100\%$$

$$= 42.3\%$$

For a 42.3 % value on the y-axis (ordinate) a value of 5.92 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.: 26).

Example: $5.92 \times 26 = 154 \text{ ng/ml}$.

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: 154 ng/ml : 7.649 = 20 nmol/l

Concentration of control samples

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

EXPECTED VALUES

IGF-I levels are highly age-dependent in children, less so in adults until the age of about 60. The normal 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. A graphic presentation is shown in Figures 3, 4 and 5. A major problem for the interpretation of IGF-I values arises from the fact, that short stature is often due to developmental delay rather than any metabolic or endocrine disorder (constitutional delay of growth and adolescence). The sharp rise in IGF-I levels during puberty may therefore cause some uncertainty as to whether or not it would be appropriate to relate measured values to chronological age. It is recommended to take the pubertal stage into account (Table 1 and Figure 6) to get a more complete picture of this situation.

Table 1: Normal range of serum IGF-I levels 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.

Pubertal Stage	Percentile			
	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

LIMITATIONS

IGF-I levels depend to a great degree on GH secretion. Diminished IGF-I values, however, do not prove GH deficiency, because a number of other factors can influence the plasma concentration of IGF-I and must therefore be taken into account in order to make a correct interpretation. IGF-I levels decrease during fasting (more than 1 day), as a result of malnutrition, malabsorption, cachexia, impaired hepatic function, or in hypothyroidism and untreated diabetes mellitus. They may also be low in chronic inflammatory disease and malignancies. IGF-I levels are high in states of accelerated sexual development. In clinical situations with hyperprolactinemia or in patients with craniopharyngioma, normal levels may be observed despite GH deficiency. In late pregnancy, IGF-I levels are moderately elevated.

Tab.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.

Age	Percentile													
	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
8-9 y. 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
9-10 y. 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
10-11 y. 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
11-12 y. 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
12-13 y. 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
13-14 y. 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
14-15 y. 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
15-16 y. 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
16-17 y. 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
17-18 y. 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
18-19 y. 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).

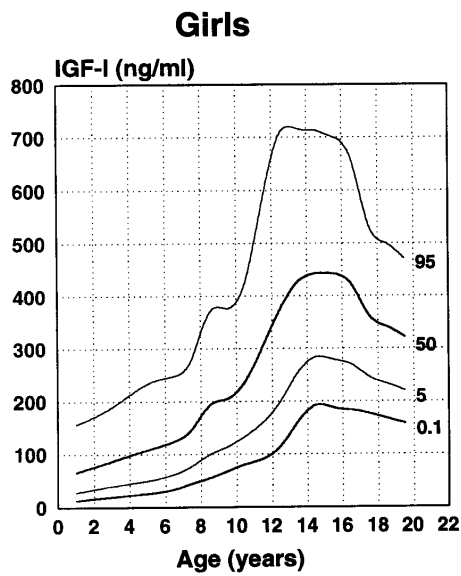


Fig. 3: Age-dependent normal range of serum IGF-I levels in girls

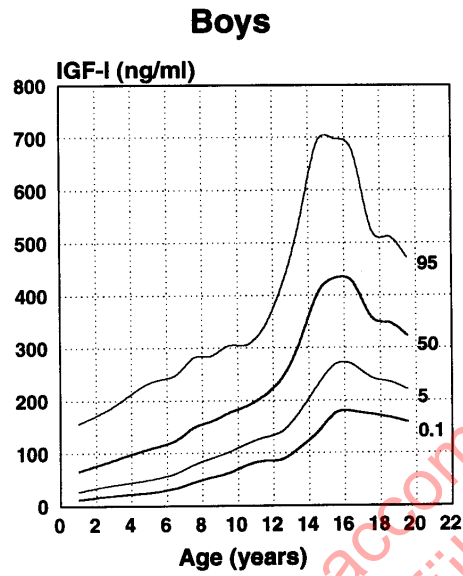


Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

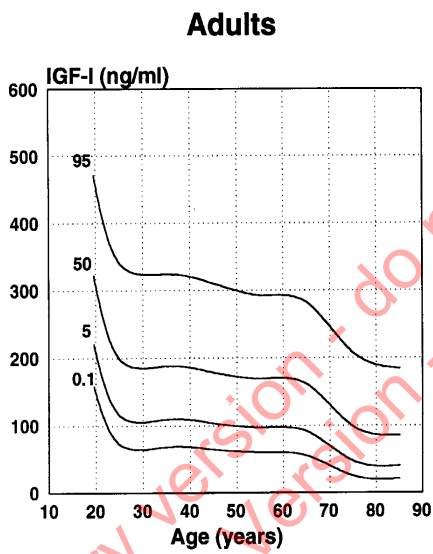


Fig. 5: Age-dependant normal range of serum IGF-I levels in adults

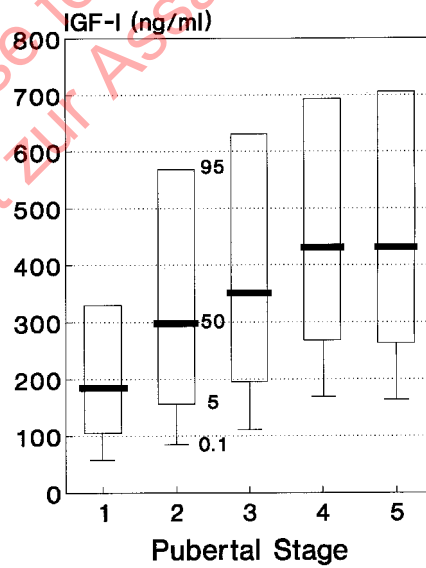


Fig 6.: Serum IGF-I levels in normal children and adolescents (7 to 17 years) according to pupertal stages. Both sexes were included.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of the radioimmunoassay for IGF-I R22 yields 0.107 ng/ml 2x SD of zero standards in 15-fold determination.

Specificity

The following materials have been evaluated for cross reactivity. 200ng/ml solutions of each substance have been analysed in this Radioimmunoassay.

	IGF-II	Insulin	Proinsulin
Reactivity [%]	0.021	0.061	0.024

Reproducibility

Intra-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	8	179.6	4.5	2.5
Sample 2	8	55.8	2.6	4.6
Sample 3	8	140	4.7	3.4

Inter-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	18	186.1	8.31	4.5
Sample 2	18	54.8	2.71	4.9
Sample 3	18	137.9	8.61	6.2

Linearity

Dilution:	Sample 1 (calculated, ng/ml)	Dilution:	Sample 2 (calculated, ng/ml)
1:20	175.9	1:20	126.4
1:23	175.6	1:22	130.2
1:26	172.8	1:26	137.6
1:30	183.6	1:30	133.8
1:35	186.0	1:35	143.6
1:40	188.2	1:40	136.0
AV / 1SD	180.9 / 6.04	AV / 1SD	134.6 / 5.99

AV = Average Value , SD = Standard Deviation

Recovery

The **recovery** of the recombinant IGF-I yielded in a buffer matrix 100%. In different human-sera the recovery was on average 102.5% % of the hypothetical expected amount.

100ng/ml IGF-I added	Serum 1	Serum 2
Recovery [%]	105.9%	98.2%

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Exemplary version - do not use for assay development!
Exemplarische Version - nicht zur Assayentwicklung benutzen!

Exemplary version - do not use for assay accomplishment!
Exemplarische Version - nicht zur Assaydurchführung benutzen!

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Exemplarische Version - nicht zur Assaydurchführung benutzen!

Summary of the assay

Reagent Preparation:	Reconstitution:	Dilution:
Capture Antibody (R)	in 5.5 ml Assay Buffer (A)	
Specific Antibody (S)	in 5.5 ml Assay Buffer (A)	
Tracer (C)	in 11 ml Assay Buffer (A)	
Control (M)+(N)	in 100 µl A.dest. each	1:26 with DB

Dilute Plasma/Serum Samples with Dilution Buffer (DB) e.g. 1:26.
If very low IGF-I levels are expected, see page 14.

Assay Procedure for Double Determinations

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	Standards	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, 350 rpm

(Alternatively: Incubate overnight without shaking (i.e., at least 15 hours) at 2 - 8°C, mix tubes before incubation see page 16)

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

Add 500 µl of reagent A (Assay Buffer) to the tubes

Aspirate the liquid completely (see above)

Count the radioactivity of all tubes.