BioVendor
Research
and Diagnostic Products

Human IGF-I ELISA

(Insulin Like Growth Factor-I)

Cat. No.: RMEE20

IVD CE

TECHNICAL FEATURES+APPLICATIONS

- High Specificity for IGF-I
- Correct measurement of IGF-I in **non-extracted** samples
- No physical separation of IGF-I from IGF-binding proteins required
- Elimination of interference by IGF-binding proteins through excess IGF-II
- 98,7 % recovery of recombinant IGF-I leads to correct absolute values
- Precise measurement of very low IGF-I levels: **sensitivity** of **0.09 ng/ml**
- Small sample volume requirement, thus ideal for young patients
- Inter- and Intra-assay variance: 6.8 and 6.7%

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 in the sample interference comes up (see example Figure 1):

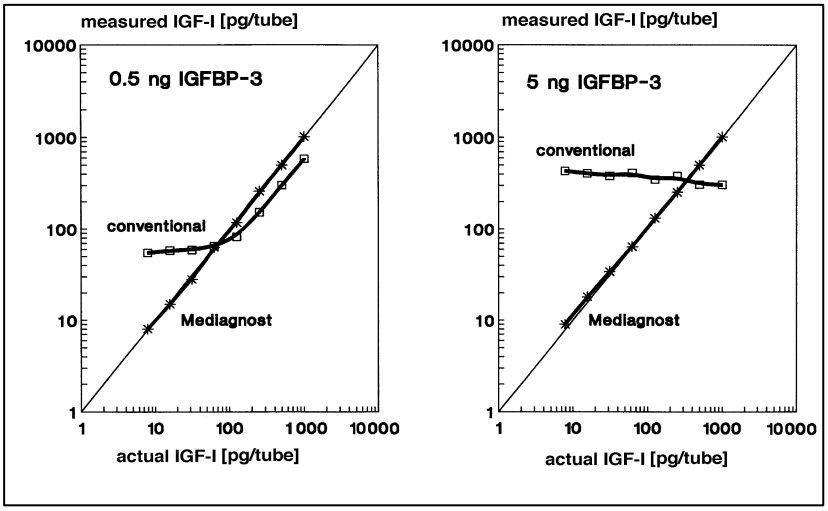


Figure 1. Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□)and by the IGFBP-blocked assay (*).

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.

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.

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. Subnormal 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).

Scientific Use

IGF-I is present in low concentrations in various body fluids and in conditioned cell culture media of many cell lines. However, the determination of IGF-I in these specimens is particularly difficult due to the presence of IGFBPs ususally in excessive amounts. This explaines why conventional assays, in which IGFBPs are not removed, result in incorrect IGF-I values, which reflect more the present amount of IGFBP rather than the exact concentration of IGF-I (Figure 1.) (15,21). The low IGF-I concentrations require often additional efforts after the extraction procedure to concentrate the extract for obtaining a satisfactory sensitivity. The IGFBP-blocked IGF-I ELISA avoids these problems and allows the simple, correct and sensitive IGF-I determination in numerous samples at the same time.

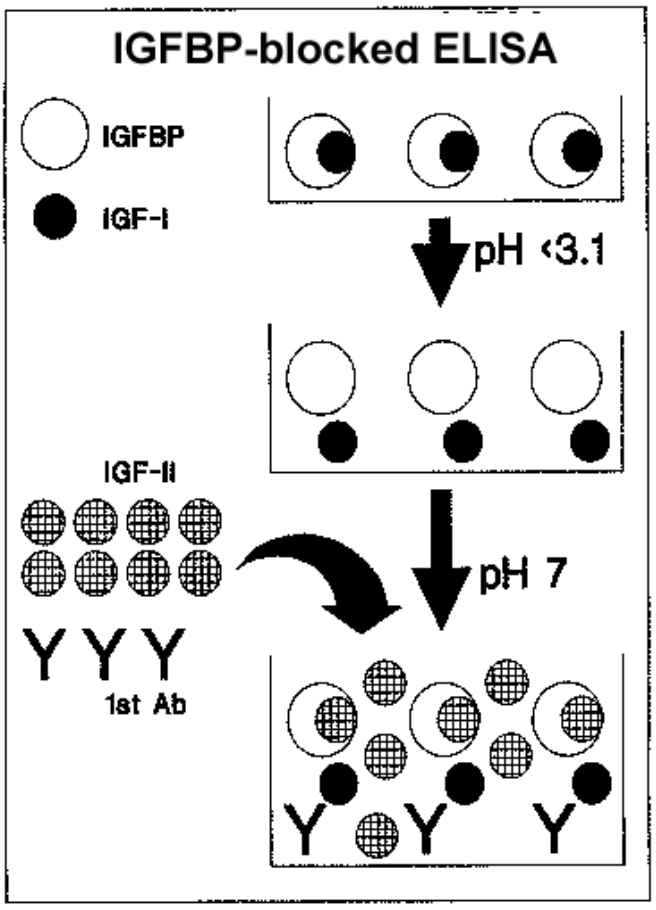
INTENDED USE

This ELISA kit is suitable for the scientific and diagnostic measurement of IGF-I in human serum or plasma, other human body fluids or conditioned media of human cell lines. Due to the high cross-reactivity 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, horse, donkey, goat, dog, cat, rabbit and guinea pig, however for rat, mouse and chicken derived samples the kit is not suited.

PRINCIPLE

The ELISA for IGF-I RMEE20 is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. The IGF-I in the sample binds to the immobilized first antibody on the microtiter plate, the biotinylated and Streptavidin-Peroxidase conjugated second specific anti-IGF-I-Antibody binds in turn to the immobilized IGF-I. In the closing substrate reaction the turn of the colour will be high specific catalysed, quantitatively depending on the IGF-I-level of the samples.



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 wells, by this the pH-value will be neutralized. After neutralization of the samples, the excess IGF-II occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of resulting 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, the excess of IGF-II does not disturb the interaction with IGF-I.

The test runs like a conventional ELISA using a Streptavidin-Peroxidase-Enzyme Conjugate.

Figure 2: Principle of the IGFBP blocked IGF-I ELISA

PERFORMANCE CHARACTERISTICS AND VALIDATION

The standards of the ELISA RMEE20 are **recombinant IGF**-I in concentrations of **1,5,15,30 and 50 ng/ml**, respectively the assay range covers –at recommended normal sample dilution- the **range from 21 to 1050 ng/ml**. By varying the sample dilution this can be adapted to the special individual requirements.

Sensitivity

The **analytical sensitivity** of the ELISA RMEE20 yields **0.09 ng/ml** (2 SD of zero standard in 17fold determination).

The **Inter**- and **Intra-Assay variation** coefficients are less than 6.8% and 6.7 % respectively. Exemplary determinations are representend in the Table 1 and 2.

Table 1: Inter-Assay-Variation

	Mean value (ng/ml)	Standard deviation	VC %
Sample 1	174	11.79	6.79
Sample 2	494	11.11	2.25
Sample 3	142	8.68	6.11

Table 2: Intra-Assay-Variation (n=18)

	Mean value (ng/ml)	Standard deviation	VC %
Sample 1	144.8	9.63	6.65
Sample 2	140.79	7.15	5.08
Sample 3	138.02	7.86	5.69

Linearity

Dilution:	**Sample 1** (recalculated, ng/ml)	**Dilution:**	**Sample 2** (recalculated, ng/ml)
1:10	137.2	1:10	439.1
1:20	133.5	1:20	500.2
1:40	133.6	1:40	499.2
1:80	134.6	1:80	490.5
1:160	134.4	1:160	494.5
1:320	135.7	1:320	526.4
		1:640	463.69
AV / SD / VC%	134.8 / 1.4 / 1.04	**AV / SD / VC%**	487.6/ 28.2 / 5.79

AV = Average Value , SD = Standard Deviation; VC = Coefficient of Variation

Recovery
In different human sera the **recovery** was on average **98.67%** of the hypothetical expected amount.

The calibration of the IGF-I ELISA with regard to the WHO International Reference Standard preparation of IGF-I, **NIBSC Code 87/518** (25-27), yields a conversion factor of 1.075. The results must be multiplied by 1.075 to express IGF-I measurements according to the present international standard preparation (its effective content by respective unit definition).
The **recovery** of the International Standard 87/518 in ELISA yielded on average 93%, with the values between 88.6-99.3%.

Validation
The IGF-I ELISA was developed in accordance with the IGF-I RIA. The clinical validation of the radioimmunoassay was achived 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

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum samples as well as Heparin and EDTA-Plasma samples are suited.
Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination store the undiluted samples frozen -20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote) although IGF-I levels were found to be unaffected by few cycles in our experiments.
The high sensitivity of the assays allows IGF-I determinations in small sample volumes, which is limited by pipetting accuracy rather than the amount of IGF-I. For most of the determinations (Serum- or Plasma-Samples and no extreme values expected) the **dilutions** of **1:10 to 1:50** in **Sample Buffer PP** should be suited. If necessary –depending on the expected IGF-I level- it is possible to use higher or lower dilution in **Sample Buffer PP.**

Generally, a dilution of **1:21 is very well suited for serum or plasma samples**. IGF-I concentration in other body fluids or cell culture supernatants however could differ strongly.
Suggestion for dilution protocol:
Please pipette 200 µl **Sample Buffer PP** in PE/PP-Tubes (applicaton of a multi-stepper is recommended in larger series); subsequently add 10 µl serum or plasma samples (dilution 1:21). After mixing use 20 µl of this dilution per determination.

REAGENTS PROVIDED

1)	MTP	**Microtiter plate,** ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with anti-human IGF-I Antibody, packed in a laminate bag.
2)	CAL	**Standards A-E**, lyophilised, contain recombinant human IGF-I. Standard values are **between 1- 50 ng/ml** (1, 5, 15, 30 and 50 ng/ml) IGF-I and have to be reconstituted in **250 µl (each) in Sample Buffer PP**. After using store the reconstituted standards in the original flasks as soon as possible at –20°C. When using the standards anew, please thaw them rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay. 20 µl per well are used in the assay
3)	BUF X	**Dilution Buffer VP**, 25 ml, ready for use, please use for the dilution of Antibody Conjugate AK and Enzyme Conjugate EK.
4)	BUF X	**Sample Buffer PP** 25 ml, ready for use, please use for reconstitution of Standards and Control Serum KS and for dilution of samples and Control Serum KS.
5)	Control	**Control Serum KS**, 100 µl, lyophilised, contains human serum and has to be reconstituted in **100 µl Sample Buffer PP**. The reconstituted Control Serum KS must be stored in the original flask as soon as possible at –20°C after using. When using anew, please thaw it rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay. The IGF-I target value concentration and the respective range is given on the vial label. The dilution of the Control Serum KS should be according to the dilution of the respective samples.
6)	Ab	**Antibody Conjugate AK**, 120 µl, 101fold Concentrate, contains the biotinylated anti-IGF-I antibody. Dilute before use **1:101 in Dilution Buffer VP** (e.g. 100 µl AK + 10 ml VP) and use 80 µl for each well in the assay.
7)	CONJ	**Enzyme Conjugate EK**, 140 µl, 101fold Concentrate, contains horseradisch-peroxidase conjugate to streptavidin. Dilute before use **1:101 in Dilution Buffer VP** (e.g. 120 µl AK + 12 ml VP) and use 100 µl for each well in the assay.
8)	WASHBUF 20x	**Washing Buffer (WP),** 50 ml, 20 X concentrated solution.
9)	SUBST	**Substrate (S),** 12 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
10)	H_2SO_4	**Stopping Solution (SL),** 12 ml, ready for use, 0.2 M sulphuric acid, Caution acid!
11)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes (100 and 200 µl) Micropipettes and multichannel pipettes with disposable plastic tips

Distilled or Deionized water for dilution of the Washing Buffer (WP)

Vortex-mixer

Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)

Timer (120 min. range)

Reservoirs (disposable)

Plate washer and plate shaker (recommended)

Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620nm (or ≥590 nm)
Foil welding device for laminate bags (recommended)

REAGENT PREPARATION

Room temperature incubation means: Incubation at 20 - 25°C.

The microtiter plate and all reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

The Standards **A – E** and **Control Serum KS** are reconstituted with the **Sample Buffer PP** provided in the Kit. It is recommended to keep the reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

Use the **Dilution Buffer VP** for the dilution of **Antibody** and **Enzyme Conjugate concentrates AK and EK.**

The shelf life of the components after opening is not affected, if used appropriately. Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C. Reconstituted Components (**Standards A – E** and **Control Serum KS**) should be stored at -20°C (or below). Freezing extends the expiry at least 2 months. When using the standards anew, please thaw them rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay.

The 1:20 diluted Washing Buffer **WP** is only 4 weeks stable. Please dilute only according to daily requirements.
Before use, all kit components should be brought to room temperature. **Precipitates, possible in buffers, should be dissolved before use through mixing and warming**.

The **Substrate Solution S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

When performing the assay, the Standards **A-E,** Control Serum **KS** and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times the diluted Enzyme Conjugate **EK** as well as the succeeding **Substrate Solution S** should be added to the plate in the same order and in the same time interval as the samples. **Stop Solution SL** should be added to the plate in the same order as the Substrate Solution **S**

STORAGE CONDITIONS

The microtiter plate wells and all undiluted reagents are stable until the expiry date if stored in the dark at 2-8°C. Store the unused seal strips and microtiter wells together with the desiccant at 2° to 8°C.

The Substrate Solution (S), stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

Store the unused seal stripes of the microtiter plate together with the desiccant at 2-8°C. Reconstituted components (**Standards A – E** and **Control Serum KS**) should be stored at -20°C (or below). Freezing extends the expiry at least 2 months. When using the Standards or Control Serum KS anew, please thaw them rapidly but gently (no temperature rise over the room temperature and no powerful vortexing), 3 of these freezing-thawing cycles showed no influence on the assay.

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only. For professional use only.
Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature WILL affect the absorbance** readings of the assay. However, values for the patient samples will not be affected.

Reagents from Kits with different lot numbers should not be mixed. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of human and/or animal origin. Source of human serum for the Control Serum provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) 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.

Stop Solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38 Irritating to eyes and skin
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1 After contact with skin, wash immediately with plenty of water

S36/37 Wear suitable protective clothing and gloves.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps. Some reagents contain ≤0.095% Kathon and/or ProClin 950 as preservatives. In case of contact with eyes or skin, flush immediately with water.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

General first aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

ASSAY PROCEDURE

NOTES: All determinations (Standards, Control Serum and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

It is recommended to use the microtiterplate shaker, if this is not available, please use the alternative procedure (see below).

1) Add **80 µl 1:101 diluted Antibody Conjugate AK** in **all** wells used

2) pipette in positions A1/2 **20 µl Sample Buffer PP**

3) Pipette in positions B1/2 **20 µl of the Standard A (1 ng/ml)**

Pipette in positions C1/2 **20 µl of the Standard B (5 ng/ml)**,

Pipette in positions D1/2 **20 µl of the Standard C (15 ng/ml)**,

Pipette in positions E1/2 **20 µl of the Standard D (30 ng/ml)**,

Pipette in positions F1/2 **20 µl of the Standard E (50 ng/ml)**.

To control the correct accomplishment of the assay **20 µl** of the 1:21 (or in respective dilution ratio of the samples) in Sample Buffer diluted **Control Serum KS** can be pipetted in positions G1/2 .

Pipette **20 µl** each of the diluted samples (e.g. dilute 1:21 with Sample Buffer **PP**) In the rest of wells, according to your requirements.

4) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at ≥350 rpm)

5) After incubation aspirate the contents of the wells and wash the wells 3 times **250 µl Washing Buffer WP** / well.

6) Following the last washing step pipette **100 µl** of the **1:101 diluted Enzyme Conjugate EK** in each well.

7) Cover the wells with sealing tape and incubate the plate for **30 Minutes** at **room temperature** (if possible shake ≥350 rpm).

8) After incubation wash the wells 3 times with Washing Buffer **WP** as described in step 5.

9) Pipette **100 µl** of the **Substrate Solution S** in each well.

10) Incubate the microtiter plate for **15 minutes in the dark** at **room temperature**.

11) Stop the reaction by adding **100 µl Stopping Solution SL** to all wells.

12) Measure the absorbance within **30 minutes at 450 nm (Reference filter ≥ 590 nm; e.g. 620 nm)**.

ALTERNATIVE PROCEDURE

Without Shaking

If there is no microtiter plate shaker available, the sample might not be mixed optimally within the well. In this case, it is recommended to dilute the samples **1:21 in Sample Buffer PP**. Then, further dilute these prediluted samples **outside of the wells** 1:5 in the 1:101 prediluted Antibody Conjugate **AK**. Subsequently add 100 µl of this external diluted sample per well. The following incubation without shaking should then take place at room temperature (20-25°C) as described in the assay procedure, however the incubation time has to be prolonged to 2 hours (instead of 1 hour). Please perform all other steps of the assay procedure according to the assay protocol given above.

CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.20, and the absorbance of standard E should be greater than 1.00.
Samples, which yield higher absorbance values than **Standard E**, are beyond the standard curve, for reliable determinations such samples should be retested at a higher dilution.

Establishing the Standard Curve

The standards provided contain the following concentration of recombinant hIGF-I:

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	1	5	15	30	50

1) Calculate the **mean absorbance** (MA) value for the blank from the duplicated determination (well A1/A2).
2) Subtract the mean absorbance (MA) of the blank from the mean absorbances of all other values.
3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis on semi-log paper (lin-log).
4) Recommendation: Calculation of the standard curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **Non-linear regression**, a higher-grade polynomial or four parametric logistic (4-PL) lin-log curve fit are suitable for the evaluation. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.
5) The concentration in ng/ml of the samples can be calculated by multiplication with the respective dilution factor.

EXPECTED NORMAL 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 5 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 4 and Figure 6) to get a more complete picture of this situation.

LIMITATIONS OF PROCEDURE

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.

Appendix

Table 4: Normal range of serum IGF-I levels (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.

	Percentiles			
Pubertal Stage	**0,1.**	**5.**	**50.**	**95.**
1	61	105	186	330
2	105	156	298	568
3	113	196	352	631
4	171	268	431	693
5	165	263	431	706

Table 5: Serum levels of IGF-I in healthy subjects at various ages. Individuals between 8 and19 years of age were classified according to gender, as the pubertal peak occurs almost 2 years earlier in girls than in boys

	Percentiles													
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

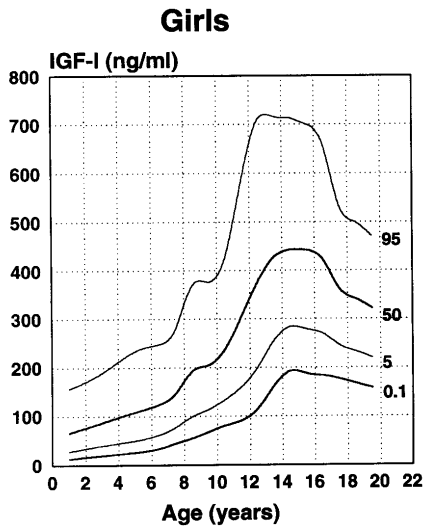


Fig. 3: Age-dependent normalrange of serum IGF-I levels in girls

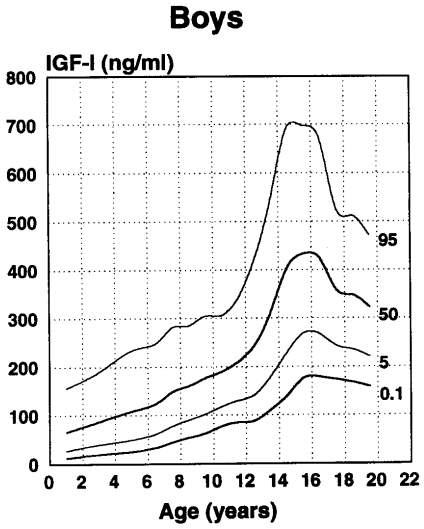


Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

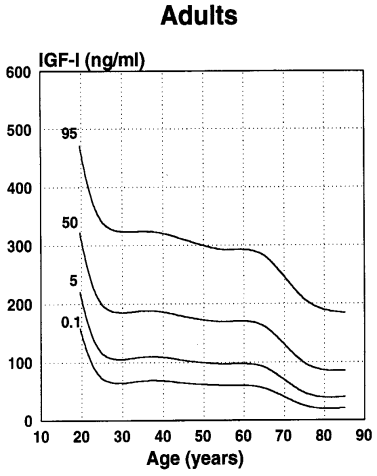
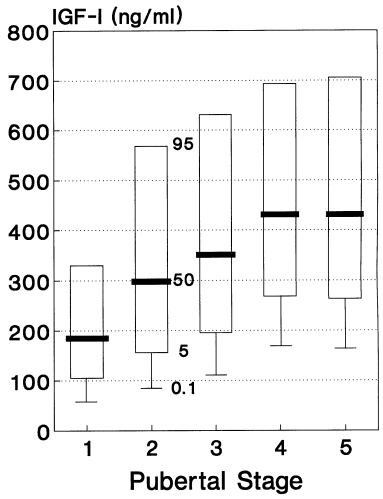


Fig. 5: Age-dependant normal range of serum IGF-I levels in adults



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