Instructions for Use

IGF-I ELISA

Enzyme Immunoassay for the quantitative determination of Insulin-like Growth Factor-I (IGF-I) in human serum and plasma.

REF **MD58011**

Σ **96**

 **2-8°C**

EU: IVD CE

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Read entire protocol before use

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
- Calibration against the WHO International Reference Standard preparation of IGF-I, **WHO NIBSC 02/254**
- 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**
- Inter- and Intra-assay variance: 6.8 and 6.7%
- Small sample volume requirement, thus ideal for young patients.
- For clinical application: **only one dilution** necessary
- Simple method for the correct measurement of IGF-I in samples with low IGF-I and high IGFBP

INTENDED USE

Measurement of human IGF-I in human serum and plasma samples.

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):

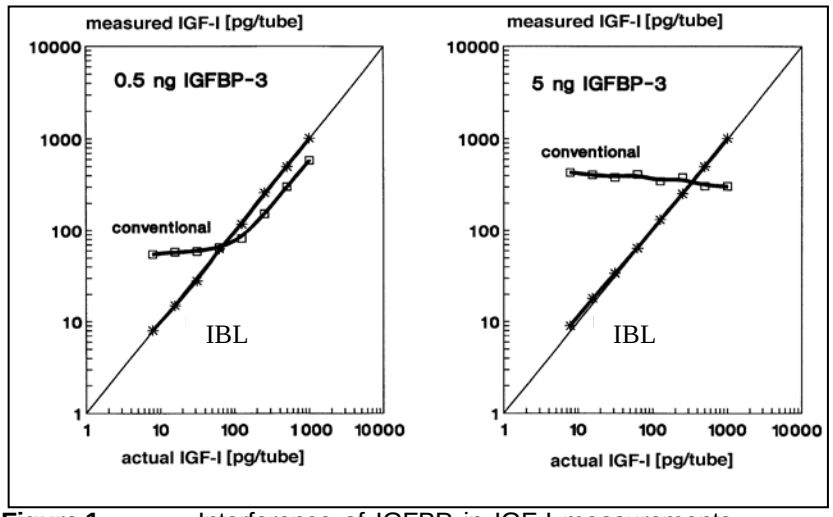


Figure 1. Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□)and by the IGFBP-blocked assay (⁎).

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 lay 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

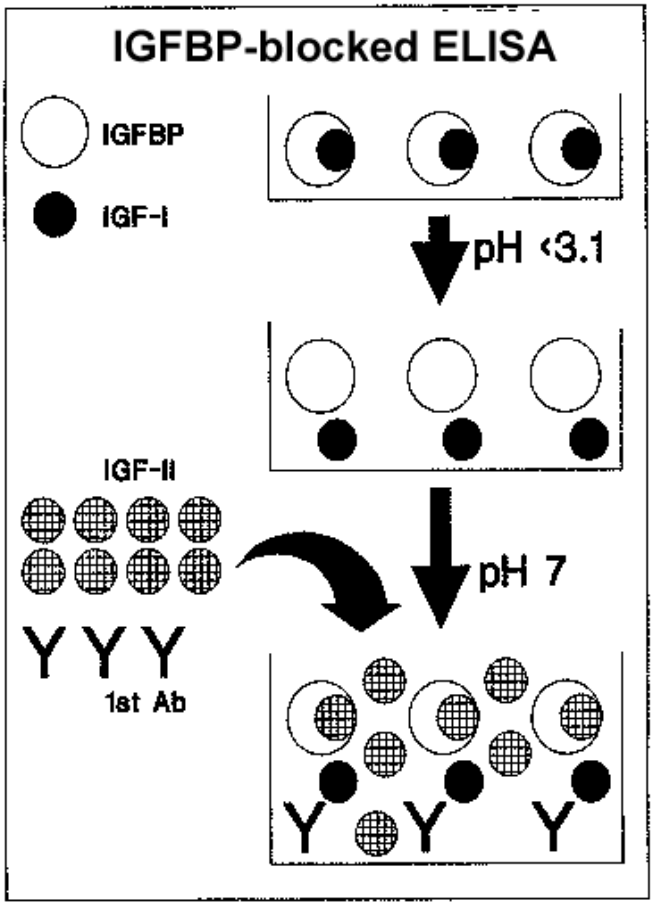
IGF-I is present in low concentrations in various body fluids and in conditioned cell culture media of many cell lines. However, the determination of IGF-I in these specimens is particularly difficult due to the presence of IGFBPs ususally in excessive amounts. This explaines why conventional assays, 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, in cerebrospinalfluid and 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 **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 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 (**Sample 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 are **recombinant IGF**-I in concentrations of **2,5,15,30 and 50 ng/mL**, respectively the assay range covers –at recommended normal sample dilution- the **range from 42 to 1050 ng/mL**. By varying the sample dilution this can be adapted to the special individual requirements.

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

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

The **Inter**- and **Intra-Assay variation** coefficients are **less than 6.8% and 6.7 %** respectively. Exemplary determinations are represented 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

Table 3: Linearity (typical results of two different sera)

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

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

Validation

The IBL IGF-I ELISA was developed in accordance with the IBL 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-, EDTA- and Citrat-Plasma samples are suited. Possible dilution of the sample by the anticoagulant must be considered.
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. 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** should be suited.

Generally, a dilution of **1:21 is well suited for serum or plasma samples**.

Suggestion for dilution protocol:
Please pipette 200 µL **Sample Buffer** in PE/PP-Tubes (application 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 within max. 2 h**.

If necessary – depending on the expected IGF-I level- it is possible to use higher or lower dilution in **Sample Buffer.** Attention: Serum or Plasma samples must be diluted at least 1:10 in Sample Buffer in order to achieve the sufficient sample acidification.

IGF-I concentration in other body fluids or cell culture supernatants however could differ strongly.

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 A LYO CAL B LYO CAL C LYO CAL D LYO CAL E LYO	**Standards A-E**, lyophilised, contain recombinant human IGF-I. Standard values are **between 2 50 ng/mL** (2, 5, 15, 30 and 50 ng/mL) IGF-I and have to be reconstituted in **500 µL (each) in Sample Buffer**. 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)	SAMPLEBUF	**Sample Buffer** 25 mL, ready for use, please use for reconstitution of Standards and Controls and for dilution of samples and Controls
4)	CONTROL 1 LYO CONTROL 2 LYO	**Control 1, 2**, 500 µL, lyophilised, contain human serum and has to be reconstituted in **500 µL Sample Buffer**. The reconstituted Control Sera must be stored in the original flask as soon as possible at –20°C after using. When using 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 IGF-I target value Concentrations / acceptable ranges see QC Certificate. The dilution of the Control Sera should be according to the dilution of the respective samples.
5)	ANTIBODY	**Antibody Conjugate**, 9 mL, ready for use, contains the biotinylated anti-IGF-I antibody. use 80 µL for each well in the assay. **ATTENTION: READY FOR USE!**
6)	ENZCONJ	**Enzyme Conjugate**, 12 mL, ready for use, contains horseradisch-peroxidase conjugate to streptavidin, use 100 µL for each well in the assay. **ATTENTION: READY FOR USE!**
7)	WASHBUF CONC	**Washing Buffer, 50 mL, 20X concentrated** solution. **Washing Buffer** has to be diluted 1:20 with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 mL) into a graduated flask and fill up with A.dest. to 1000 mL). Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
8)	TMB SUBS	**TMB Substrate,** 12 mL, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
9)	TMB STOP	**TMB Stopping Solution,** 12 mL, ready for use, 0.2 M sulphuric acid, Caution acid!
10)		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes and multichannel pipettes with disposable plastic tips
Distilled or deionized water for dilution of the Washing Buffer)
Vortex-mixer
Microtiter plate shaker (350 rpm)
Microtiter plate washer (recommended)
Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
Polyethylene PE/Polypropylene PP tubes for dilution of samples

TECHNICAL NOTES

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

The Standards **A – E** and **Controls** are reconstituted with the **Sample Buffer** 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.

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 1+2**) should be stored at -20°C (or below). Freezing extends the expiry at least 3 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** 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**. **Temperature WILL affect the absorbance** readings of the assay. However, values for the patient samples will not be affected

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

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

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtitre plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must become adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/or false values, excessive shaking may result in high optical densities and/or false values.

Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided washing buffer diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

The danger of handling with potentially infectious material must be taken into account.

When using an automatic microtitre plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamicly swinging out the microtitre plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non fuzzy absorbent tissue.

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, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.
(**Standards A – E** and **Controls**) should be stored at -20°C. Freezing extends the expiry at least 2 months. When using the Standards or Controls 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.
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.
Appropriate precautions and good laboratory practice must be observed during storage, use and disposal of the components of the kit. The disposal of the kit components shall be in accordance with local regulations.
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. The components should be treated as potentially infectious.

Human serum:
Controls contains material of human 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) antibodies. 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.

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.

Following components contain < 0.01% (w/w) **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** as preservative: ANTIBODY, ENZCONJ, WASHBUF, SAMPLEBUF

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1 S28.1	After contact with skin, wash immediately with plenty of water

Following components contain < 0.01% **2-Methyl-4-isothiazolin-3-one solution** as preservative ANTIBODY, ENZCONJ, SAMPLEBUF

R34	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice

TMB SUBS **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

TMB STOP **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.

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 Sera and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

1) Add **80 µL Antibody Conjugate** in **all** wells used

2) Pipette in positions A1/2 **20 µL Sample Buffer**

3) Pipette in positions B1/2 **20 µL of the Standard A (2 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 1** and **2** can be pipetted in positions G1/2 and H1/2.

   Pipette **20 µL** each of the diluted samples (e.g. dilute 1:21 with Sample Buffer) 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 **5 times 300 µL Washing Buffer**/ well.

6) Following the last washing step pipette **100 µL** of the **Enzyme Conjugate** in each well.

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

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

9) Pipette **100 µL** of the **Substrate Solution**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** to all wells.

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

CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of standard E should be above 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	2	5	15	30	50
nmol/ L	0.26	0.66	1.96	3.92	6.54

The Conversion factor of ng/mL in nmol/L is 0.13074

1) Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).
2) Subtract the mean absorbance 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.
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. **A higher-grade polynomial,** or **four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
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 /Anhang

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 / Perzentilen					
Pubertätsstadium	**0,1.**	**5.**	**50.**	**95.**	**Pubertal Stage**
1	61	105	186	330	**1**
2	85	156	298	568	**2**
3	113	196	352	631	**3**
4	171	268	431	693	**4**
5	165	263	431	706	**5**

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

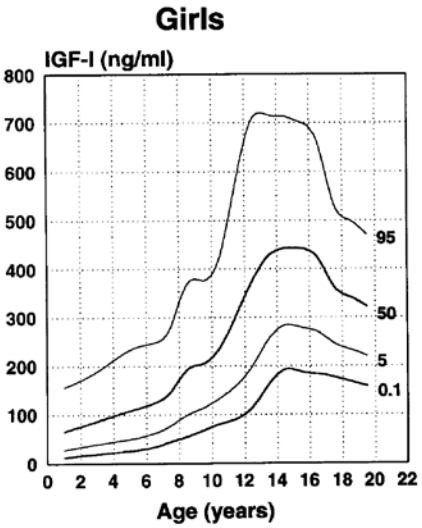


Fig. 3: Age-dependent normalrange of serum IGF-I levels in girls

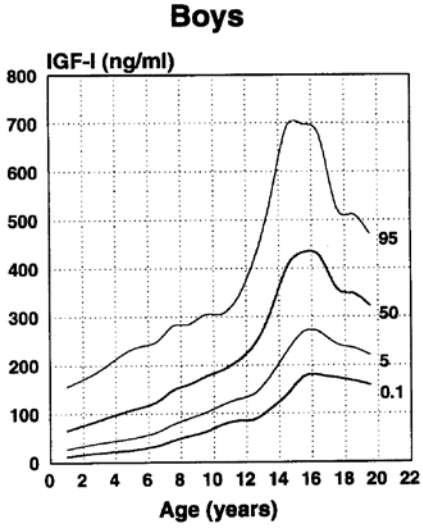


Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

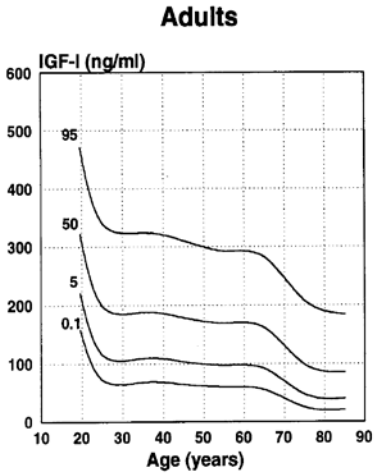


Fig. 5: Age-dependant normal range of serum IGF-I levels in adults

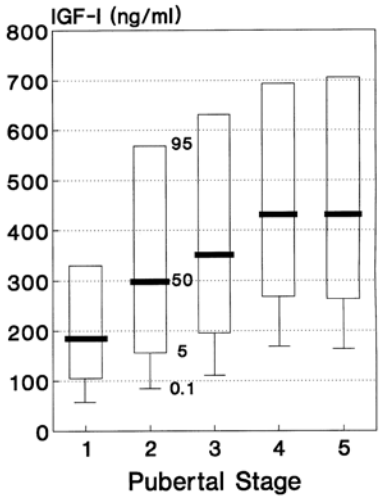


Fig 6.: Serum IGF-I levels in normal children and adolescents (7 to 17 years) according to pubertal stages. Both sexes were included.

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SUMMARY – IGF-I ELISA

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in **Sample Buffer**	**500 µL**
Control 1	Reconstitution in **Sample Buffer**	**500 µL**
Control 2	Reconstitution in **Sample Buffer**	**500 µL**
Wash Buffer	dilute in **A. dest.** (eg. total volume of 50 mL in a graduated flask and fill up to 1000 mL)	**1:20**
Sample and Controls: dilute 1:21 in Sample Buffer, mix immediately, incubate max. 2h. **Use 20 µL** for each well in the assay.		
Before conducting the assay equilibrate all **reagents to room temperature.**		

Assay Procedure for Double Determinations:

Pipette	Reagent	Position
80 µL	Antibody Conjugate	in **all** wells used
20 µL	Sample Buffer (blank)	A1 and A2
20 µL	Standard **A** (**2 ng/mL**)	B1 and B2
20 µL	Standard **B** (**5 ng/mL**)	C1 and C2
20 µL	Standard **C** (**15 ng/mL**)	D1 and D2
20 µL	Standard **D** (**30 ng/mL**)	E1 and E2
20 µL	Standard **E** (**50 ng/mL**)	F1 and F2
20 µL	Control **1**	G1 and G2
20 µL	Control **2**	H1 and H2
20 µL	Diluted Samples	following wells
Cover the wells with the sealing tape.		

Incubation: 1 h at RT, 350 rpm

5x 300 µL	Aspirate the contents of the wells and wash **5x** with **300 µL Wash Buffer**	each well
100 µL	**Enzyme Conjugate**	each well

Incubation: 30 min at RT, 350 rpm

5x 300 µL	Aspirate the contents of the wells and wash **5x** with **300 µL Wash Buffer**	each well
100 µL	**TMB Substrate**	each well

Incubation: 15 min in the dark RT

100 µL	**Stop Solution**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

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