IGF-I ELISA Assay Kit

Reagents for Quantitative Determination of

Insulin-like Growth Factor-I

(IGFBP blocked)

Product-Code: E20
(96 Determinations)

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

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INTENDED USE

The Eagle Biosciences IGF-I ELISA Assay Kit is for research use only and not for diagnostic procedures. This kit is intended for the quantitative determination of human IGF-I concentrations in serum and plasma.

SUMMARY AND EXPLANATION

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):

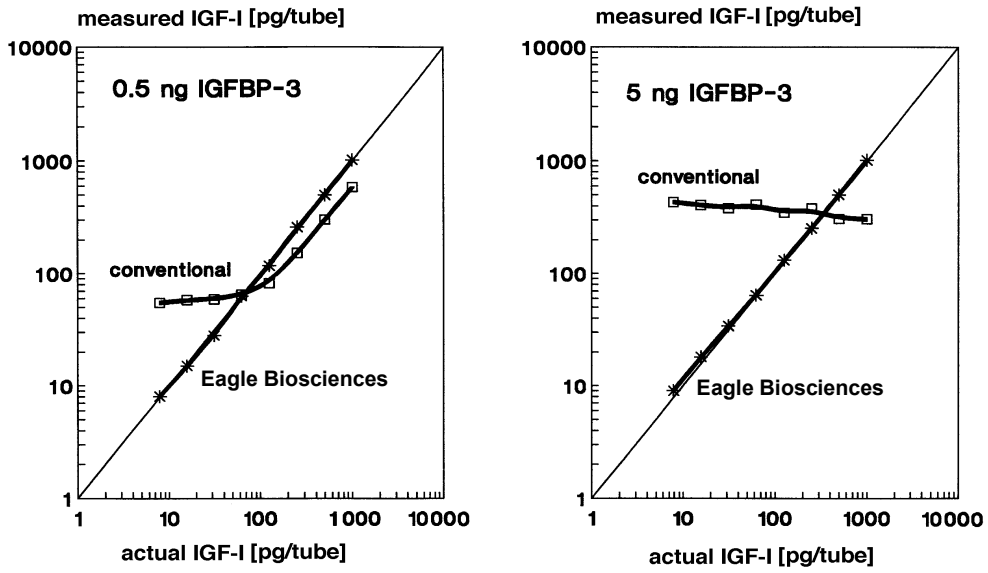

Figure 1. Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□) and by an IGFBP-blocked assay (*).

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.

Principal of the Assay

In order to dissociate IGF-I from the IGFBPs, the samples must be diluted in an acidic buffer (**Sample Buffer PP)** (Figure 2). The diluted samples are then pipetted into the assay wells. The IGF-I antiserum is dissolved in a buffer, which is able to neutralize the acidic samples. After the IGF-I antibody solution has neutralized the samples, the present excess IGF-II occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of the 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 of the first antibody with IGF-I.

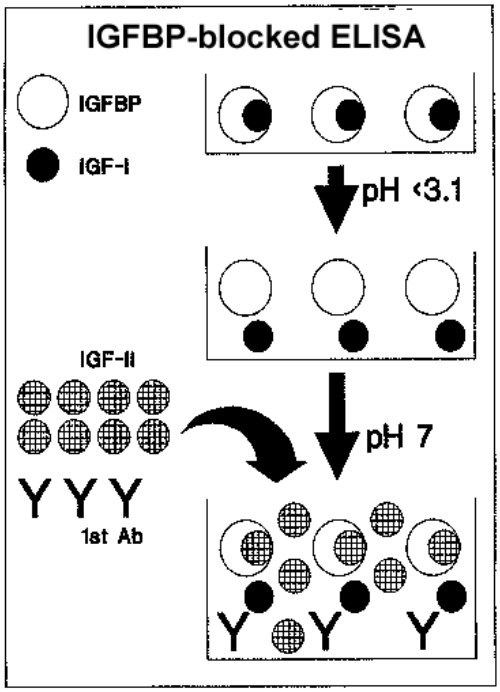

Figure 2.: Principle of the IGFBP-blocked IGF-I ELISA

The Eagle Biosciences ELISA for IGF-I is a so-called Sandwich-Assay using two specific and high affinity antibodies. The IGF-I in the samples binds to the first antibody coated on the microtiterplate, the second specific anti-IGF-I-antibody binds in turn to the immobilised IGF-I. The second antibody is biotinylated, the subsequently incubated Streptavidin-Peroxidase-Enzyme Conjugate will bind to it, and thus in the final substrate incubation step colour development will be catalysed quantitatively depending on the IGF-I-level of the samples. The Standards of this ELISA are prepared from recombinant IGF-I in concentrations of 2, 5, 15, 30 and 50 ng/ml.

WARNINGS AND PRECAUTIONS

For Research Use only. Not for use diagnostic procedures.

This IGF-I ELISA Assay Kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Eagle Biosciences, Inc. will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

Human Serum
Following components contain human serum: **Control Serum KS and KS2**
Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious.

Reagents A-E, AK, EK, VP, WP
Contain as preservative a mixture of **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

Substrate Solution (S)
The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stopping Solution (SL)
The Stopping solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

1.1 General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove 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: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

REAGENTS PROVIDED

1)	MTP	**Microtiter plate,** ready for use, 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)	STD	**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 PP**. After using store the reconstituted standards in the original flasks as soon as possible at –20°C (-4°F). 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 PP	**Sample Buffer PP, 25 ml**, ready for use, please use for reconstitution of Standards and Controls and for dilution of Samples and Controls
4)	Control	**Control Serum KS1 and KS2, 500 µl,** lyophilised, contain human serum and has to be reconstituted in **500 µl Sample Buffer PP**. The reconstituted Control Sera must be stored in the original flask as soon as possible at –20°C (-4°F) 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 and the respective ranges are given on the vial labels. The dilution of the Control Sera should be according to the dilution of the respective samples.
5)	Ab	**Antibody Conjugate AK, 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)	CONJ	**Enzyme Conjugate EK, 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 20x	**Washing Buffer (WP), 50 ml, 20 X concentrated solution**. **Washing Buffer (WP), 50 ml, 20X concentrated** solution. **Washing Buffer (WP)** 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)	SUBST	**Substrate (S), 12 ml,** ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
9)	H_2SO_4	**Stopping Solution (SL), 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

- Distilled or demineralised water for dilution of the Washing Buffer WP
- Micropipettes and multichannel pipettes with disposable plastic tips
- Vortex-mixer
- Microtiter plate Shaker (350 rpm)
- Microtiter plate washer (recommended)
- Microtiter plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm.
- Polyethylen PE/Polypropylen PP tubes for dilution of samples

TECHNICAL NOTES

- Bring all reagents to room temperature 68-77°F (20 - 25°C) before use.
- **Wash Solution**: Add distilled/deionized water to the complete contents of the (20x) concentrated Wash Solution (50 ml) into a graduated flask and fill to a final volume of 1000 ml, or dilute 1:20 only according to requirements. The diluted Washing Buffer is stable for max. 4 weeks at 35.6-46.4°F (2-8°C).
- For the reconstitution of the lyophilised components (Standards A - E and Control Sera KS1 & KS2) Sample Buffer PP should be used. It is recommended to keep 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 incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter 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 microtiter 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 dynamically swinging out the microtiter 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 **microtiterplate wells** and all undiluted reagents are stable until the expiry date, if stored in the dark at 35.6-46.4°F (2-8°C).
- Store the microtiterplate wells sealed together with the desiccant at 35.6-46.4°F (2-8°C).
- The **Substrate Solution S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.
- Reconstituted components (**Standards A – E** and **Control Sera KS1 & KS2**) should be stored at –4°F (-20°C). Freezing extends the expiry at least 2 months. When using the Standards or Control Sera KS1 or KS2 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.
- Precipitates, possible in buffers, should be dissolved before use through mixing and warming.

- **Room temperature incubation means: Incubation at 68-77°F (20-25°C).**

SPECIMEN COLLECTION, PREPARATION AND STORAGE

The stability of IGFBP-bound IGF-I makes sample preparation simple. Blood samples may be taken at any time of the day. Whole blood should be processed within a few hours and stored frozen at –4°F (-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 98.6°F (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 Preparation

- Serum samples as well as Heparin-, EDTA- and Citrat-Plasma samples are suited. Possible dilution of the sample by the anticoagulant must be considered.
- Serum or plasma samples should be diluted depending on the expected values 1:10 -1:50 with **Sample Buffer PP**. Generally, in case of serum or plasma specimens a dilution of **1:21** is very well suited. IGF-I concentration in other body fluids or cell culture supernatants could differ strongly.
- Suggestion for dilution protocol:

  Pipette 200 µl **Sample Buffer PP** in PE/PP-Tubes (applicaton of a multi-stepper is recommended in larger series); add 10 µl serum or plasma samples (dilution 1:21). After mixing use 2 × 20 µl of this dilution in the assay within max. 2h.

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.

When performing the assay, the **Standards**, **Control Sera** and the samples should be pipette 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**.

1) Add **80 µl 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 (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 PP** diluted **Control Sera KS1&KS2** can be pipetted in positions G1/2 and H1/2.
   Pipette **20 µl** each of the diluted sample (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** 68-77°F (20-25°C) (shake at 350 rpm).
5) After incubation aspirate the contents of the wells and wash the wells 5 times **300 µl Washing Buffer WP** / well.
6) Following the last washing step pipette **100 µl** of the **Enzyme Conjugate EK** in each well.
7) Cover the wells with sealing tape and incubate the plate for **30 minutes** at **room temperature** 68-77°F (20-25°C) (shake at 350 rpm).
8) After incubation wash the wells 5 times with **Washing Buffer WP** as described in step 5.
9) Pipette **100 µl** of the **Substrate Solution S**.
10) Incubate the plate for **15 minutes in the dark** at **room temperature** 68-77°F (20-25°C).
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**).

QUALITY CONTROL

The handling of potentially infectious material must comply with Good Laboratory Practice (GLP). GLP requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP or other applicable federal, state or local standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

CALCULATION OF RESULTS

Assay Characteristics and Validation

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. The Eagle Biosciences, Inc. IGF-I enzymeimmunoassay is calibrated against the International Reference Standard preparation of IGF-I, **WHO NIBSC Code 02/254** (25, 26)

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 these samples should be re-tested with a higher dilution.

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

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	**2**	**5**	**15**	**30**	**50**

1) Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).
2) Subtract the means absorbance of the blank from the mean absorbances of the standards and of the samples.
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 standard curve should be done by using a computer programme, 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 IGF-I **concentration of the sample in ng/ml** can be calculated by **multiplication** with the respective **dilution factor.**

Example of Typical Standard Curve

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

Standard	A	B	C	D	E
ng/ml	**2**	**5**	**15**	**30**	**50**
extinction	0.07	0.18	0.59	1.06	1.58

EXEMPLARY VALUES

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

	Percentile			
Pubertal Stage	**0.1th**	**5th**	**50th**	**95th**
1	61	105	186	330
2	85	156	298	568
3	113	196	352	631

4	171	268	431	693
5	165	263	431	706

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

		Percentile													
Age		**0.1**	1	**5**	10	**20**	30	**40**	50	**60**	70	**80**	90	**95**	99
0-2 y.		**13**	20	**28**	34	**43**	50	**58**	66	**75**	87	**102**	128	**156**	220
2-4 y.		**20**	29	**40**	48	**59**	68	**77**	87	**98**	111	**129**	159	**189**	260
4-6 y.		**26**	36	**50**	59	**73**	85	**96**	108	**122**	138	**160**	196	**233**	320
6-7 y.		**34**	46	**62**	72	**87**	99	**111**	124	**138**	155	**176**	212	**248**	332
7-8 y.		**45**	60	**78**	90	**107**	121	**134**	148	**163**	181	**205**	243	**281**	364
8-9 y.	boys	**54**	71	**90**	102	**119**	133	**146**	160	**175**	192	**214**	250	**284**	362
	girls	**55**	75	**99**	115	**137**	156	**174**	193	**214**	239	**271**	324	**376**	496
9-10 y.	boys	**63**	82	**102**	115	**133**	148	**162**	176	**191**	209	**232**	269	**304**	379
	girls	**68**	89	**114**	130	**152**	170	**187**	205	**224**	247	**276**	323	**369**	469
10-11 y.	boys	**77**	96	**117**	130	**148**	162	**176**	189	**203**	220	**241**	274	**305**	370
	girls	**81**	106	**134**	153	**178**	199	**219**	239	**261**	287	**321**	374	**426**	539
11-12 y.	boys	**85**	106	**129**	144	**163**	179	**194**	209	**225**	244	**267**	304	**339**	413
	girls	**91**	123	**160**	185	**220**	248	**276**	305	**337**	374	**424**	503	**581**	758
12-13 y.	boys	**8811**	112	**141**	159	**184**	204	**223**	243	**264**	289	**321**	371	**419**	525
	girls	**6**	155	**201**	231	**274**	309	**342**	377	**415**	460	**519**	614	**707**	914
13-14 y.	boys	**111**	143	**179**	203	**235**	261	**286**	311	**339**	371	**412**	477	**540**	677
	girls	**163**	207	**256**	287	**329**	364	**395**	428	**463**	504	**556**	637	**716**	884
14-15 y.	boys	**140**	182	**229**	260	**303**	337	**370**	404	**441**	484	**539**	625	**691**	896
	girls	**193**	236	**284**	314	**353**	385	**414**	443	**474**	510	**556**	628	**713**	832
15-16 y.	boys	**176**	221	**269**	299	**340**	372	**402**	433	**466**	504	**552**	626	**697**	849
	girls	**187**	231	**279**	309	**350**	382	**412**	442	**474**	512	**559**	632	**700**	845
16-17 y.	boys	**178**	221	**267**	296	**335**	366	**395**	424	**455**	491	**537**	607	**673**	814
	girls	**183**	225	**270**	298	**336**	366	**394**	422	**452**	486	**530**	597	**660**	792
17-18 y.	boys	**173**	207	**243**	265	**294**	317	**337**	358	**380**	405	**436**	484	**527**	618
	girls	**176**	210	**246**	268	**297**	320	**341**	362	**384**	409	**441**	488	**533**	624
18-19 y.	boys	**167**	201	**235**	256	**285**	307	**327**	347	**368**	393	**423**	469	**512**	600
	girls	**167**	199	**233**	254	**281**	302	**322**	341	**362**	385	**414**	458	**499**	583
19-20 y.		**158**	189	**220**	240	**265**	285	**304**	322	**341**	363	**391**	433	**471**	550
20-30 y.		**72**	92	**115**	130	**150**	167	**182**	198	**215**	235	**261**	302	**340**	425
30-40 y.		**68**	87	**109**	123	**142**	158	**173**	188	**204**	223	**248**	287	**324**	404
40-50 y.		**64**	82	**103**	116	**135**	150	**164**	178	**194**	212	**235**	272	**310**	385
50-60 y.		**60**	77	**97**	110	**127**	142	**155**	169	**184**	201	**224**	260	**292**	369
60-70 y.		**55**	72	**91**	103	**120**	134	**147**	161	**176**	193	**215**	251	**282**	362
70-80 y.		**25**	35	**47**	55	**67**	78	**88**	98	**110**	124	**142**	173	**207**	276
>80 y.		**21**	30	**40**	47	**58**	67	**76**	85	**95**	108	**125**	153	**184**	245

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

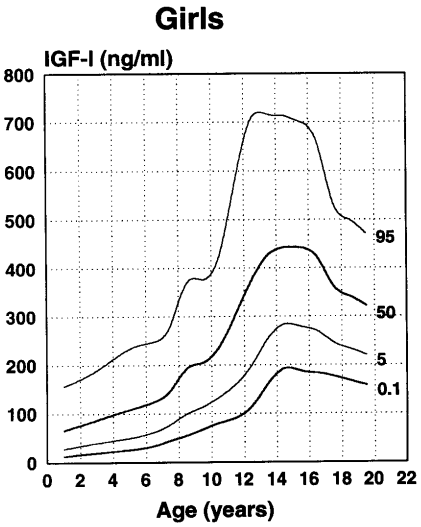

Fig. 3: Age-dependent normalrange of serum IGF-I levels in girls

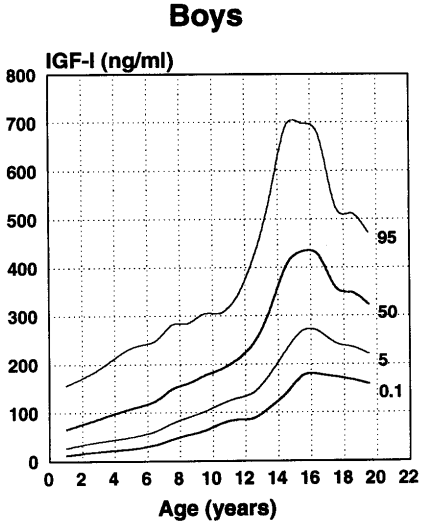

Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

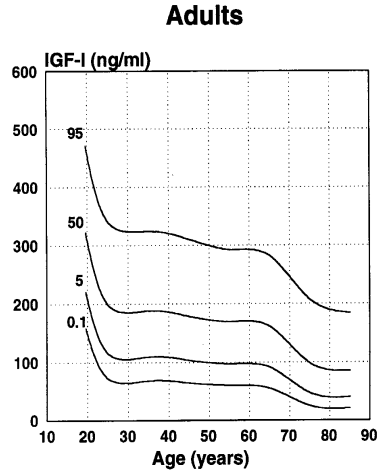

Fig. 5: Age-dependant normal range of serum IGF-I levels in adults

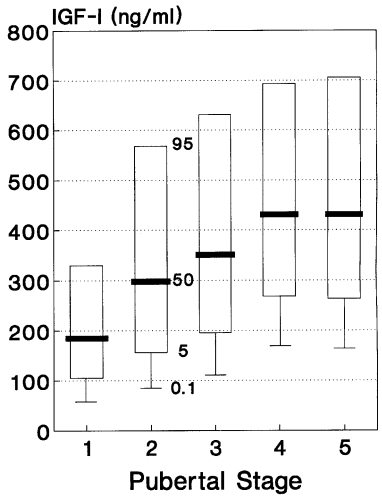

Fig 6.: Serum IGF-I levels in normal children and adolescents (7 to 17 years) according to pubertal stages. Both sexes were included.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of the enzyme immunoassay for IGF-I yields **0.11** ng/mL 2x SD of zero standards in 17-fold determination.

Specificity

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

	IGF-II	Insulin	Proinsulin	C-Peptide
Reactivity [%]	0.055	0.045	0.025	0.02

Reproducibility

Intra-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	18	144.8	9.63	6.65
Sample 2	18	140.79	7.15	5.08
Sample 3	18	138.02	7.86	5.69

Inter-Assay-Variation

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

Linearity

Dilution:	Sample 1 (calculated, ng/ml)	Dilution:	Sample 2 (calculated, 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	463.7
AV / 1SD / VC%	134.8/ 1.4/ 1.04	**AV / 1SD / VC%**	487.6/ 28.2/ 5.79

AV = Average Value , SD = Standard Deviation

Recovery

The **recovery** of the recombinant hIGF-I yielded in a buffer matrix 100%. In different human-sera the recovery was on average 98.67% (n=5) of the hypothetical expected amount.

Exemplary data

	Serum 1	Serum 2	Serum 3
IGF-I added	200 ng/ml	400 ng/ml	400 ng/ml
Recovery [%]	103.4	101.1	101.9

COMPARISON STUDIES

Eagle Biosciences, Inc. ELISA was adapted to Mediagnost IGF-I RIAs and correlates with this assay very well ($y=0.9896x+13.984$; $R^2 = 0.914$). Therefore the studies comparing the MEDIAGNOST RIA test to commercially available tests performed by Ranke et al.[28] can be used to compare this ELISA with other commercially available assays.

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SUMMARY – EAGLE BIOSCIENCES, INC. IGF-I ELISA

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in **Sample Buffer PP**	**500 µl**
Control Serum KS1	Reconstitution in **Sample Buffer PP**	**500 µl**
Control Serum KS2	Reconstitution in **Sample Buffer PP**	**500 µl**
Wash Buffer WP	dilute in **A. dest.** (e.g. total volume of 50 ml in a graduated flask and fill up to 1000 ml)	**1:20**
Sample + Control Sera KS1 and KS2: dilute 1:21 in Sample Buffer PP, 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 **AK**	in **all** wells used
20 µl	Sample Buffer **PP** (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 Serum **KS1**	G1 and G2
20 µl	Control Serum **KS2**	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 WP**	each well
100 µl	Enzyme Conjugate **EK**	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 WP**	each well
100 µl	Substrate **S**	each well

Incubation: 15 min in the dark RT

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