IGF-I ELISA

Enzyme Immunoassay for Quantitative Determination of

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

English

United States of America
For In Vitro Diagnostic Use
IVD for professional use!

IVD 2-8°C 96 wells

REF **E20**

mediagnost®
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Symbols

DIN EN ISO 15223-1

	Expiry date
	Consider instructions for use
	In Vitro Diagnostic Medical Device (For In Vitro diagnostic Use)
	Lot-Batch Number
	Manufactured by
	Catalogue Number
	Store at between
	Contains sufficient for x tests

TABLE OF CONTENTS

IGF-I ELISA E20	96 Determinations
Principle of the test	Enzyme-linked Immunoassay
Duration (incubation period)	1.75 h
Antibodies	specific, monoclonal antibody and high-affinity polyclonal antiserum
Cross reactivity with IGF-II; Insulin, C-Peptide	< 0.1%
Buffer	Ready for use and 20fold concentrate
Standard	5 single standards: 2 - 50 ng/mL, recombinant human IGF-I
Reference Material	International Standard WHO NIBSC 02/254
Assay Range	0.09 – 1050 ng/mL
Control	2 control sera, freeze-dried
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:21
Analytical sensitivity	ø 0.09 µg/L
Intra- / Interassay Variance	ø < 10 %
Reference values	Blum W.F., Schweizer R. Insulin-Like Growth Factors and Their Binding Proteins. In: Ranke MB (ed.): Diagnostics of Endocrine Function in Children and Adolescents. Basel, Karger, 2003, pp.166-199:

1 INTENDED USE

An enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of levels of IGF-I in serum or plasma. Human IGF-I measurements are used in the diagnosis and treatment of growth disorders involving the anterior lobe of the pituitary gland.

2 INTRODUCTION

Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation, differentiation and specific functions of many cell types (1-3). IGF-I is identical with Somatomedin C (Sm-C) (4) and has a molecular weight of 7649 daltons (5). Its major regulators are growth hormone (GH) and nutrition (6), although its production in specific tissues is affected by a multitude of tropic hormones and other peptide growth factors. In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven classes of IGFBPs which are known at present (7,8,22) either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II (9,10).
The interference of IGFBPs is a major problem in IGF-I measurement. 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 example Figure 1):

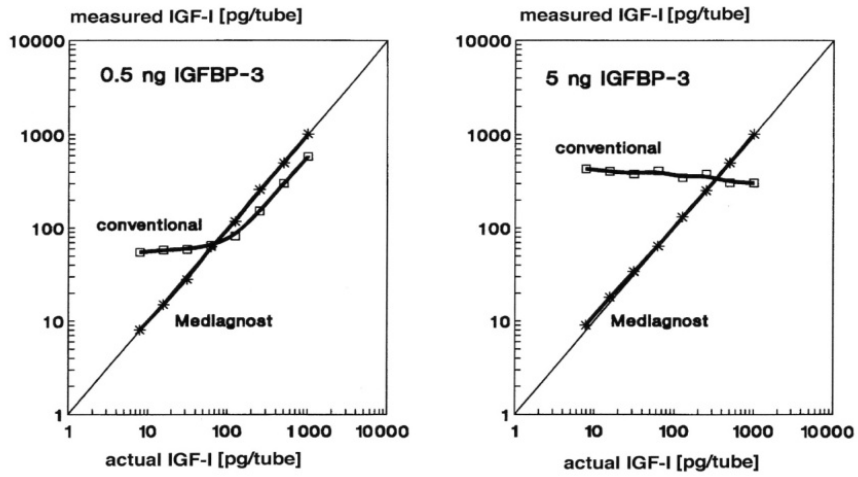

Figure 1: Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□) and by the IGFBP-blocked RIA (*).

Therefore, various techniques were applied to physically separate IGF-I from its binding proteins before measurement, including (a) size exclusion chromatography under acidic conditions, (b) solid-phase extraction and (c) acid-ethanol extraction (2,12,13). These techniques, however, are either inconvenient 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).

3 PRINCIPAL

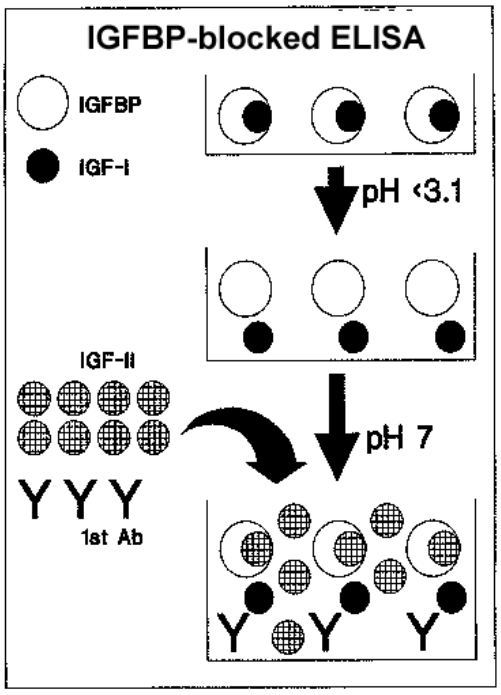

Figure 2 Principle of the IGFBP-blocked IGF-I ELISA

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.

The Mediagnost ELISA for IGF-I E20 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 the ELISA E20 are prepared from recombinant IGF-I in concentrations of 2, 5, 15, 30 and 50 ng/ml .

4 WARNINGS AND PRECAUTIONS

1. For *In Vitro Diagnostic Use*.
2. For professional use only.
3. ***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.
4. Mediagnost 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.
5. Before use, all kit components should be brought to room temperature at 20 - 25°C (68-77°F). Precipitates in buffers should be dissolved before use by thorough mixing and warming. Temperature WILL affect the absorbance readings of the assay. However, correctness of the results will not be affected.
6. Do not mix reagents of different lots. Do not use expired reagents.
7. Do not use obvious damaged or microbial contaminated or spilled material.
8. The microtiterplate contains break apart strips. Unused wells must be stored at 2 - 8 °C (35.6-46.4°F) (in the sealed foil pouch and used in the frame provided).
9. **Caution:** This kit contains material of human and/or animal origin. Source human sera for the Control Sera 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.
10. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
11. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
12. 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.
13. Mix the contents of the microtiterplate wells thoroughly to ensure good test results. Do not reuse microtiterplate wells.
14. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
15. 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. Material Safety Data Sheet is available on request.

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 sulfuric 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.

4.1 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: If swallowed the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

5 SAMPLES

5.1 Sample type

Serum and Plasma
Serum and Heparin/EDTA Plasma yield comparable values. The IGF-I levels are reduced in citrate plasma samples, because of the relatively high amount of anticoagulant.

5.2 Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions are to be avoided.

5.3 Required sample volume: **10 µL**

5.4 Sample stability

In firmly closed sample vials:

- Storage at 20-25°C (68-77°F): max. 24 hours
- Storage at -20° C (–4°F): min. 2 years
- Freeze-thaw cycles max. 3

The storage of samples over a period of 2 years at -20°C (–4°F) showed no influence on the reading. Freezing and thawing of samples should be minimized. 3 freeze-thaw cycles showed no effect on samples.

5.5 Interference

Triglyceride, bilirubin and hemoglobin in the sample do not interfere to a concentration of 100 mg/mL and 200 µg/mL or 1 mg/mL. However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

5.6 Sample dilution

- Dilution: **1:21** with **Sample Buffer PP**
- Pipette **200 µL Sample Buffer PP** in PE-/PP-Tube (application of a multi-stepper is recommended in larger series); add **10 µL sample** (dilution 1:21). After mixing use 2 x 20 µL of this dilution in the assay.
- Attention: serum and plasma samples must be diluted at least 1:10 in **Sample Buffer PP** in order to achieve sufficient acidification of the samples.
- Depending on the expected IGF-I values the samples can be diluted higher in **Sample Buffer PP**.
- Sample stability after dilution of the sample: maximum 2 hours at 20-25°C (68-77°F).

6 MATERIALS

6.1 Materials provided

The reagents listed below are sufficient for 96 wells including the standard curve.

MTP	**MTP**	**Microtiter plate,** ready for use, coated with mouse-anti-hIGF-I-antibody. Wells are	**(8x12) wells**
STD	**A-E**	**Standards**, lyophilized, (recombinant human hIGF-I), concentrations are given on vial labels and on quality	**5 x 500 µL**
CONTROL	**KS1**	**Control Serum 1,** lyophilised, (human serum), concentration is given on quality certificate.	**1 x 500 µL**
CONTROL	**KS2**	**Control Serum 2,** lyophilised, (human serum), concentration is given on quality certificate.	**1 x 500 µL**
AK	**AK**	**Antibody Conjugate,** ready for use, contains goat biotinylated anti-hIGF-I antibody**.**	**1 x 9 mL**
CONJ	**EK**	**Enzyme Conjugate,** ready for use, contains HRP (Horseradish-Peroxidase)-labelled	**1 x 12 mL**
BUF	**PP**	**Sample Buffer, ready for use**	**1 x 25 mL**
WASHBUF 20x	**WP**	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
SUBST TMB	**S**	**Substrate,** ready for use, horseradish-peroxidase- (HRP) substrate, stabilised Tetramethylbencidine.	**1 x 12 mL**
H_2SO_4	**SL**	**Stopping Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
TAPE	-	**Sealing Tape,** for covering the **microtiter plate.**	**2 x**
	-	**Instructions for use**	**1 x**
-	-	**Quality Certificate**	**1 x**

6.2 Materials required, but not provided

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer **WP (A. dest.), 950 mL.**
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

7 TECHNICAL NOTES

Storage Conditions
Store the kit at 2-8°C (35.6-46.4°F) until its expiry date. The lyophilized reagents should be stored at –20°C (–4°F) after reconstitution. Avoid repeated thawing and freezing.

Storage Life
The shelf life of the components **after initial opening** is warranted for **4 weeks**, store the unused strips and microtiter wells **airtight** together with the desiccant at 2-8°C (35.6-46.4°F) in the clip-lock bag, use in the frame provided. The **reconstituted components** standards **A-E** and Control Sera **KS1** and **KS2** must be stored at –20°C (–4°F) (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C (35.6-46.4°F).

Preparation of reagents
Bring all reagents to room temperature 20-25°C (68-77°F) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.
Do not use expired reagents. Temperature WILL affect the absorbance readings of the assay. However, correctness of the results will not be affected.

Reconstitution
The Standards **A–E** and Control **KS1 and KS2** are reconstituted with the Sample Buffer **PP**. 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.

Dilution
After reconstitution dilute the Control **KS1** and **KS2** with the Sample Buffer **PP** in the same ratio (1:21) as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20 fold concentrate with Aqua dest..

Incubation
Incubation at room temperature means: Incubation at 20 - 25°C (68-77°F). The Substrate Solution **S**, stabilised Tetramethylbencidine, is photosensitive–store and incubation in the dark.

Shaking
The incubation steps should be performed at mean rotation frequency of a microtiter plate shaker. We recommend 350 rpm. Depending on the design of the shaker, the shaking frequency should be 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.

Washing
Proper washing is of **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 **WP** diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

When using an **automatic microtiter** plate washer, the respective instructions for 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 dynamical 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.

8 ASSAY PROCEDURE

NOTES: When performing the assay, Blank, Standards **A-E**, Controls **KS1 and KS2** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate **AK,** Enzyme Conjugate **EK** and Substrate Solution **S** should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution **SL** should be added to the plate in the same order as Substrate Solution **S**. All determinations (Blank, Standards **A-E**, Control **KS1** and **KS2** 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 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** 20-25°C (68-77°F) (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** 20-25°C (68-77°F) (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** 20-25°C (68-77°F).
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**).

9 QUALITY CONTROL

The handling of potentially infectious material must comply with Good Laboratory Practice (GLP). GLP requires that controls are included in each assay. 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 (Good Laboratory Practice) 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.

Quality criteria

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**, should be re-tested with a higher dilution.

10 EVALUATION OF RESULTS

10.1 Establishing of the standard curve

The International Standard for hIGF-I, WHO NIBSC Code 02/254 was used as standard material and the following IGF-I concentrations are used.

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

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 samples and standards
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 IGF-I concentration in ng/mL of the samples can be calculated by **multiplication** with the respective **dilution factor.**

10.2 Example of a typical standard curve

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

	Blank	A	B	C	D	E
ng/mL	0.0	2	5	15	30	50
$OD_{(450\text{-}620\ nm)}$	0.00	0.088	0.299	0.985	1.727	2.543

The exemplary shown standard curve in **Figure 3 cannot** be used for calculation of your test results. You have to establish a standard curve for each test you conduct!

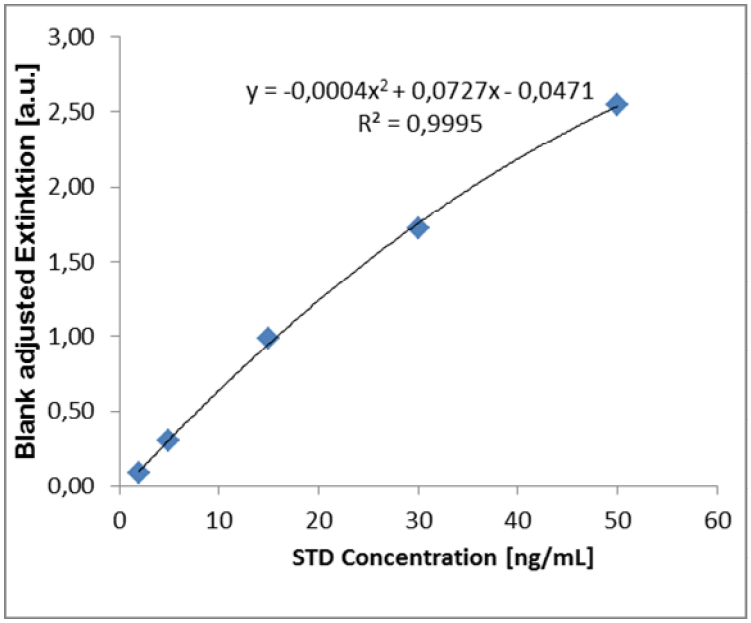

Figure 3 Examplary standard curve

10.3 Exemplary calculation of IGF-I concentrations

Sample dilution: 1:21

Measured extinction of the blank	0.0165
Measured extinction of your sample	0.2695

Your measurement program will calculate the IGF-I concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit (here: polynomial 3rd degree).

In this exemplary case the following equation is solved by the program to calculate the IGF-I concentration in the sample:

$$0.253 = -0.0004x^2 + 0.0727x - 0.0471$$
$$4.57 = x$$

If the dilution factor (**1:21**) is taken into account the **IGF-I** concentration of the undiluted sample is

$$4.57 \text{ ng/mL} \times 21 = 96 \text{ ng/mL}$$

10.4 Interpretation of results

The test results should not be the only base for therapeutic decisions. The results should be interpreted in regard to anamnesis, further clinical observations and results of other diagnostic investigations. Further, it is recommended to establish reference and cut-off values corresponding to the relevant group of patients for each laboratory. It is recommended to consider the international and national guidelines for diagnosis and treatment of growth hormone deficiency / acromegaly.

11 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.

The Mediagnost IGF-I ELISA, E20 is based on a combination of monoclonal capture and polyclonal detection antibodies. Generally, immunological assays are sensible to heterophilic antibodies and rheumatoid factors in the sample. Their influence is reduced by the assay design, but cannot be excluded completely.

12 EXPECTED VALUES

IGF-I levels are highly age-dependent in children, less so in adults until the age of about 60. The normal ranges in various age groups, which are log-normally distributed, are given in Table 2 by percentiles. These values are given only for guidance; each laboratory should establish its own reference of values for the diagnostic evaluation of patient results.
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 4, 5 and 6. 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) get a more complete picture of this situation.

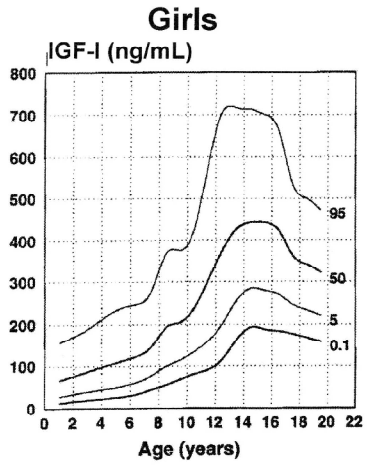

Fig. 4.: Age-dependent reference range of serum IGF-I levels in girls.

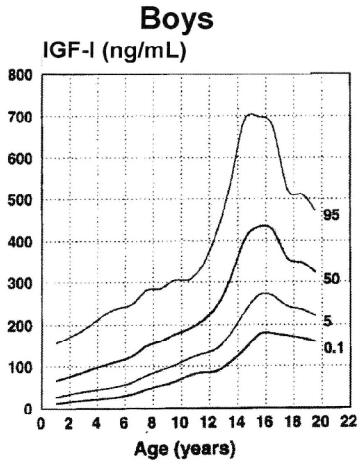

Fig. 5.: Age-dependent reference range of serum IGF-I levels in boys.

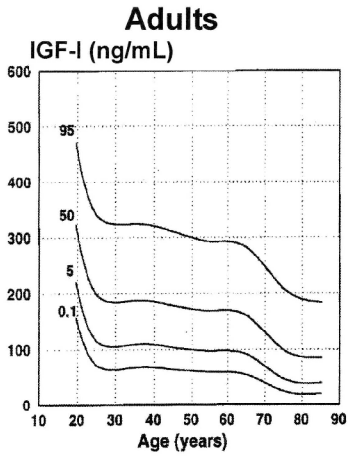

Fig. 6.: Age-dependent reference range of serum IGF-I levels in adults.

Table 1 Normal range of serum IGF-I levels given in ng/mL at different pubertal stages according to Tanner. Because no significant difference between boys and girls is observed, both sexes are combined. Only children and adolescents between 7 and 17 years of age are included.

	Percentile			
Pubertal Stage	**0.1th**	**5th**	**50th**	**95th**
1	61	105	186	330
2	85	156	298	568
3	113	196	352	631
4	171	268	431	693
5	165	263	431	706

Table 2 Serum levels of IGF-I in healthy subjects at various ages. Individuals between 8 and 19 years of age were classified according to gender, as the pubertal peak occurs almost 2 years earlier in girls than in boys.

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

Serum concentrations are given in ng/ml.

Reference values have been evaluated by Prof Blum by a radioimmunoassay identical to Mediagnost IGF-R20. Thus, the age and sex specific reference values published in Diagnostics of Endocrine Function in Children and Adolescents.
(Edited by Prof Ranke. ISBN-3-335-00496-5) can be applied to all Mediagnost IGF-I assays.

13 PERFORMANCE CHARACTERISTICS

13.1 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the Mediagnost E20 is 0.091 ng/mL on average, in 19 independent determinations values from 0.03 ng/mL to 0.2 ng/mL were found.

13.2 Specificity

The measurements of E20 cross reactivity with IGF-II, Insulin and C-Peptide. These IGF-related proteins were added to assay buffer in the indicated concentration and the solution was applied as sample without any further dilution. The concentration measured within the blank without any protein was 0.78 µg/L. Thus, neither IGF-II nor Insulin or C-Peptide are measured by the Mediagnost E20 ELISA (see table 3).

Table 3 Specificity. Cross reactivity of the test system with different IGF-I related proteins.

added C-Peptide [µg/L]	measured IGF-I [µg/L]	added Insulin [µg/L]	measured IGF-I [µg/L]	added IGF-II [µg/L]	measured IGF-I [µg/L]
500	0.73	100	0.78	1250	0.77
100	0.78	10	0.77	750	0.73
10	0.77	1	0.76	250	0.77
0	0.78	0	0.78	0	0.78

13.3 Precision

Intra-Assay Variance

Three samples have been measured six to 18 times in the same assay. The results are shown in table 4. The measured coefficient of variation (CV) is 5.81% on average.

Table 4 Intra-Assay variability

	Number of determinations	Mean value (ng/mL)	Standard deviation (ng/mL)	VC (%)
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 and Lot-to-Lot Variance

Serum samples where measured in independent assays. Exemplary results are shown in Table 5. Further, five samples were also tested repeatedly throughout four years in eight different lots. The variability on average is 8.57% (6.8 – 10.5%).

Table 5 Inter-Assay variability

	Number of determinations	Mean value [ng/mL]	Standard deviation [ng/mL]	VC [%]
Sample 1	8	81	5.34	6.56
Sample 2	16	192	12.38	6.43
Sample 3	17	498	27.52	5.53

13.4 Linearity

Linearity was tested by dilution of native sera with different IGF-I contents (Sample 1-3). The amount of measured IGF-I was recalculated and is shown in Figure 7.

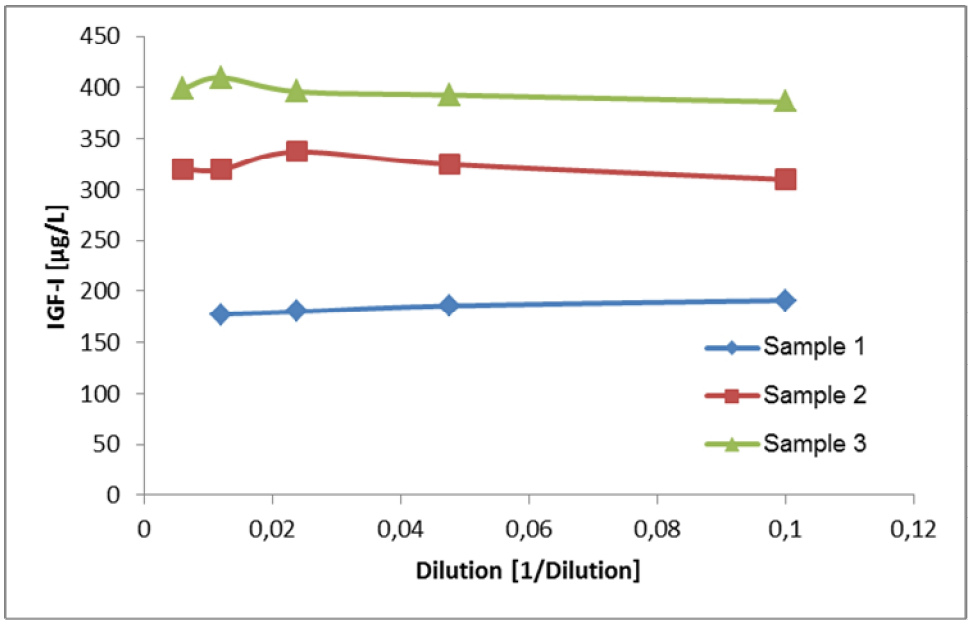

Figure 7 Linearity, recalculated IGF-I concentrations of differentially diluted samples. The minimal dilution is 1:10, the recommended dilution is 1:21.

13.5 Recovery and Accuracy

Recombinant IGF-I was added in different amounts to human serum. The IGF-I content of the so enriched samples was measured and recovery in comparison to enriched buffer calculated. Results are shown in Table 6.

Table 6 Recovery of recombinant IGF-I in human serum

IGF-I [µg/L]	Sample 1	Sample 2	Sample 3	Sample 4
Sample	138	172	133	180
+ IGF-I 200	287	372	-	-
+ IGF-I 400	-	-	539	591
% Recovery	**85**	**100**	**101**	**102**

13.6 Interference

Interference of bilirubin and triglycerides was tested by adding different amounts of these substances to human serum containing IGF-I. For comparison the same amount of buffer without any substance was also added to the serum. Table 7 demonstrates that neither bilirubin nor triglycerides exert any influence on the measurement of IGF-I in human serum.

Table 7 Interference of physiologic substances on IGF-I measurement. Human serum samples were enriched with different amounts of trigylcerides, bilirubin or hemoglobin and the recovery of IGF-I was measured. Here the relative recovery in [%] of not enriched samples is shown.

	Triglyceride 100 mg/mL	Bilirubin 200 µg/mL	Hemoglobin 10 mg/ml
Serum 1	93	90	97
Serum 2	100	101	110
Serum 3	120	120	104

Influence of binding proteins on IGF-I measurement was exemplarily elucidated with IGFBP-3.

Different amounts of IGF-I and 3 or 6 mg/L of IGFBP-3 were added to sample buffer (pH 2) and phosphate based saline buffer (pH 7.4). After a short incubation of 15 minutes at room temperature these samples were diluted and applied to the Mediagnost E20 as described in the package insert. In case of sample buffer IGFBP-3 up to 6 mg/L did not interfere with IGF-I measurement. But without acidification of the sample a strong interference of IGFBP-3 with IGF-I measurement was detected (Table 8).

Table 8 Interference of IGFBP-3 with IGF-I measurement

IGFBP-3	Sample Buffer		
	50 µg/L IGF-I	100 µg/L IGF-I	300 µg/L IGF-I
-	46.38	116.14	358.1
3 mg/L	47.33	115.83	384.15
6 mg/L	52.32	123.38	355.41
IGFBP-3	**Phosphate buffered Saline**		
	50 µg/L IGF-I	**100 µg/L IGF-I**	**300 µg/L IGF-I**
-	34.2	90.23	349.04
3 mg/L	7.4	12.16	152.14
6 mg/L	7.2	10.12	48.15

13.7 Traceability / Assay Calibration

Recombinant human IGF-I produced by E. coli and of >98% purity (SDS-PAGE, Silverstain) is used as standard within the assay. This recombinant hIGF-I devoid of methIGF-I or IGF-I variants with mismatched disulfide bonds is identical to the major authentic IGF-I form in blood. The traceability of this recombinant standard material to the international reference material of the WHO 02/254 has been proven. Results are published by Burns C et al. in Growth Horm IGF Res. 2009 Oct;19(5):457-62. Epub 2009 Mar 20. Mediagnost E20 ELISA is coded by 14c.

The reference material includes **8.5 µg/ampoule** IGF-I measured by amino acid analysis and HPLC. Mediagnost E20 immunoassay (assay No. 14c) measures **11.55 µg/ampoule**. The mean of all tested immunoassays is 11.61 µg/ampoule.

Thus, Mediagnost results are comparable to other immunological tests for measurement of IGF-I and can easily be transformed to WHO 02/254 (25, 26) by a factor of **0.735**.

14 ASSAY COMPARISON

Mediagnost E20 IGF-I was compared with the Mediagnost R20 IGF-I. 196 serum samples were measured in both assays and an excellent coefficient of correlation was shown with r = 0.95. Additionally, the Mediagnost IGF-I ELISA E20 was compared with an Enzyme-Immunoassay of other commercially available IGF-I test and a correlation of R^2>0.9 was shown.

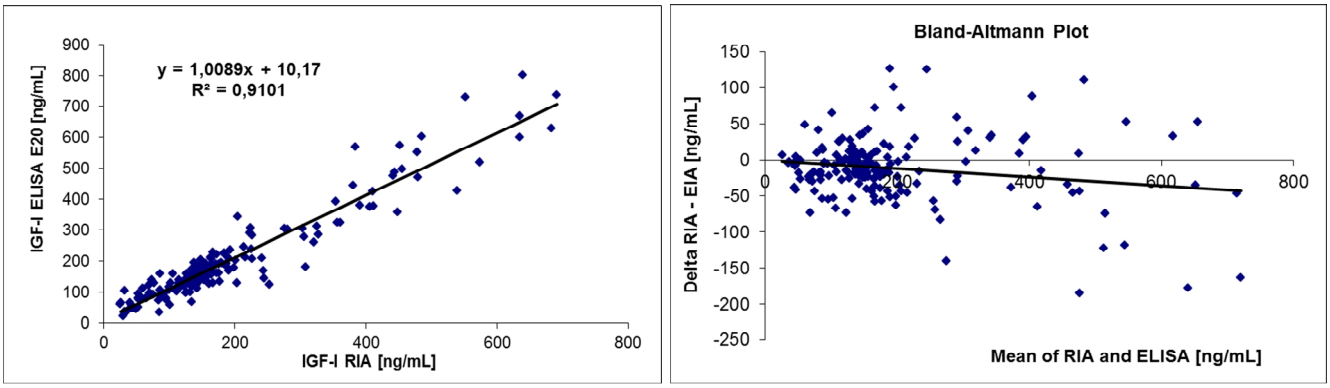

Figure 8 Assay Comparison Mediagnost RIA R20 and Mediagnost ELISA E20

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16 SUMMARY OF ASSAY PROCEDURE

Preparation of reagents		**Reconstitution:**	**Dilution**
A-E	**Standards**	in **500 µL** Sample Buffer **PP**	-
KS1	**Control Serum 1**	in **500 µL** Sample Buffer **PP**	**1:21** with Sample Buffer **PP**
KS2	**Control Serum 2**	in **500 µL** Sample Buffer **PP**	**1:21** with Sample Buffer **PP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**
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 assay procedure bring all reagents to room temperature **20-25°C (68-77°F).**			

Assay procedure in double determination			
Pipette	**Reagents**		**Position**
80 µL	Antibody Conjugate **AK**		in **all** wells used
20 µL	Sample Buffer **PP** (Blank)		A1/A2
20 µL	Standard **A** (**2 ng/mL**)		B1/B2
20 µL	Standard **B** (**5 ng/mL**)		C1/C2
20 µL	Standard **C** (**15 ng/mL**)		D1/D2
20 µL	Standard **D** (**30 ng/mL**)		E1/E2
20 µL	Standard **E** (**50 ng/mL**)		F1/F2
20 µL	Control Serum **KS1**	(1:21 diluted)	G1/G2
20 µL	Control Serum **KS2**	(1:21 diluted)	H1/H2
20 µL	Sample	(1:21 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.			
Sample-Incubation: 1 h at 20-25°C (68-77°F), 350 rpm			
5x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WP/ well**		In each well
100 µL	Enzyme Conjugate **EK**		In each well
Cover the wells with the sealing tape.			
Incubation: 30 Minutes at 20-25°C (68-77°F) 350 rpm			
5x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WP/ well**		In each well
100 µL	Substrate Solution **S**		In each well
Incubation: 15 Minutes in the Dark at 20-25°C (68-77°F)			
100 µL	Stopping Solution **SL**		In each well
Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.			