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Instructions for use

IGF-1 ELISA

REF **ME E-0500**

IVD CE

IGF-1 ELISA

Introduction

Intended Use

The **IGF-1 ELISA** is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Insulin-like Growth Factor 1 (IGF-1) in serum.

Summary and Explanation

Clinical Significance
Insulin-like growth factor I (IGF-1) is a polypeptide of 70 amino acids (7650 daltons), and is one of a number of related insulin-like growth factors present in the circulation .The molecule shows approximately 50% sequence homology with proinsulin and has a number of biological activities similar to insulin. The peptide is growth hormone (GH) dependent to a high degree, but there is growing evidence of GH-independent secretion. IGF-1 has numerous growth-promoting effects, including mitogenic effects and the promotion of cartilage sulphation. It may also be implicated in the rate of bone turnover.
Almost all (>95%) of serum IGF-1 circulates bound to specific IGF binding proteins, of which six classes (IGF-BPs 1 - 6) are now recognized. BP3 is thought to be the major binding protein of IGF-1, forming a ternary complex of 140 000 daltons with IGF-1 and an acid-labile subunit.

Clinical Applications
The measurement of serum IGF-1 is of recognized value in children with growth disorders and in the diagnosis and monitoring of acromegaly. IGF-1 concentrations change with age, nutritional status, body composition and growth hormone secretion.
A single basal IGF-1 determination is useful in the assessment of short stature in children and in nutritional support studies of acutely ill patients. For the diagnosis of acromegaly, a single IGF-1 determination is considered more reliable than a random GH determination.

PRINCIPLE of the test
The IGF-1 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
Patient samples, standards and controls are acidified and neutralized prior to the assay procedure.
The microtiter wells are coated with a monoclonal antibody directed towards an antigenic site on the IGF-1 molecule.
The pre-treated sample is incubated at room temperature with Conjugate (biotinylated IGF-1). The wells are washed and then incubated with Enzyme Complex (Streptavidin-HRP-Complex).
After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of IGF-1 in the patient sample.

WARNINGS AND PRECAUTIONS
1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
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. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.

15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

REAGENTS

Reagents provided

HCL **ME E-0519 1 M *HCl***

1 vial, 3 mL, ready to use; for sample acidification.

NEUTR-SOLN **ME E-0587** ***Neutralization Buffer Solution***

1 vial, 3 mL, ready to use; for neutralization of samples.

96 **ME E-0531** ***Microtiterwells***

12x8 (break apart) strips, 96 wells; Wells coated with a anti-IGF-1 antibody (monoclonal).

Standards & Controls

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**ME E-0501**	Standard 0	0 ng/ml	1 ml
STANDARD B	**ME E-0502**	Standard 1	5 ng/ml	1 ml
STANDARD C	**ME E-0503**	Standard 2	10 ng/ml	1 ml
STANDARD D	**ME E-0504**	Standard 3	50 ng/ml	1 ml
STANDARD E	**ME E-0505**	Standard 4	150 ng/ml	1 ml
STANDARD F	**ME E-0506**	Standard 5	300 ng/ml	1 ml
STANDARD G	**ME E-0507**	Standard 6	600 ng/ml	1 ml
CONTROL 1	**ME E-0551**	Control Low	Refer to vial labels or QC-datasheet for expected value and acceptable range!	1 ml
CONTROL 2	**ME E-0552**	Control High		1 ml

The standards are calibrated against the International Reference Reagent for IGF-1, NIBSC code: 87/518
conversion: 1ng/mL x 0.13 = nmol/L.
Contain non-mercury preservative.

CONJUGATE **ME E-0540** ***Enzyme Conjugate***

1 vial, 14 mL, ready to use; biotinylated IGF-1; contains non-mercury preservative.

ENZYME **ME E-0515** ***Enzyme Complex***

1 vial, 20 mL, ready to use; Streptavidin HRP Complex; contains non-mercury preservative.

SUBSTRATE **FR E-0055** ***Substrate Solution***

1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).

STOP-SOLN **FR E-0080** ***Stop Solution***

1 vial, 14 mL, ready to use; contains 0.5M H_2SO_4.
Avoid contact with the stop solution. It may cause skin irritations and burns.

WASH- CONC 40x **FR E-0030** ***Wash Solution***

1 vial, 30 mL (40X concentrated); see „Preparation of Reagents".

Note: Additional *Standard 0* for sample dilution is available on request.

Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- 1.5 mL Reaction Cups (e.g. from Eppendorf) for Sample Preparation (Acidification and Neutralization).
- Timer
- Semi logarithmic graph paper or software for data reduction

Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 6 weeks if stored as described above.

Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

Damaged Test Kits

In case of any severe damage of the test kit or components, the supplier has to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN

Serum can be used in this assay.
NOTE: In plasma significantly reduced values were observed.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Specimen Storage and Preparation

Specimens should be assayed immediately.
Specimens held for a longer time (at least one year) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Acidification and Neutralization of Patient Samples, Standards and Controls

1. Pipette **200 µL Sample, Standard or Control** in 1.5 mL-Reaction caps (E.g. Eppendorf-Caps).
   Please note: The standards should be acidified and neutralized too, according to the procedure described below
2. Add **20 µL 1 M HCl.**
3. Mix and incubate for 15 minutes.
4. For Neutralization add **20 µL Neutralization Buffer** to all caps and mix the solution.
   A pH check and correction of pH is not necessary.
   Immediately (within 10 minutes) continue with ELISA test procedure.

ELISA Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **50 µL** of each acidified and neutralized ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3.	Dispense **100 µL *Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4.	Incubate for **120 minutes** at room temperature.

5.	Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets. **Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6.	Dispense **150 µL** *Enzyme Complex* into each well.
7.	Incubate for **60 minutes** at room temperature.
8.	Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9.	Add **100 µL** of ***Substrate Solution*** to each well.
10.	Incubate for **30 minutes** at room temperature.
11.	Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
12.	Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 600 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and <u>cannot</u> be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	1.94
Standard 1 (5 ng/mL)	1.69
Standard 2 (10 ng/mL)	1.49
Standard 3 (50 ng/mL)	0.93
Standard 4 (150 ng/mL)	0.56
Standard 5 (300 ng/mL)	0.44
Standard 6 (600 ng/mL)	0.33

<u>Expected normal values</u>

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Children at pre-puberty (age 3-8 years): 20 – 250 ng/mL
Smaller children with a low body weight could show values under 50 ng/mL.

Children at puberty (age 11 – 16 years): 130 – 600 ng/mL
Adults after puberty: 150 – 350 ng/mL

Note: The IGF-1 level could slightly decrease at age.

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Quality Control

Good laboratory practice 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.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit.
The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor.

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 1.29 – 600 ng/mL.

Analytical Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 (S0) and was found to be 1.29 ng/mL

Reproducibility

Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	29.04	4.72
2	20	58.94	6.62

Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	28.06	7.22
2	12	57.52	7.79

Recovery

Samples have been spiked by adding IGF-1 solutions with known concentrations in a 1:1 ratio.
The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added Concentration 1:1 (v/v) (ng/mL)	Measured Conc. (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
	0.0	28.9		
	300.0	294.3	314.4	93.6
1	150.0	152.4	164.4	92.7
	75.0	77.0	89.4	86.1
	25.0	38.5	39.4	97.6
	0.0	56.4		
	300.0	334.8	328.2	102.0
2	150.0	191.3	178.2	107.4
	75.0	100.0	103.2	96.9
	25.0	46.8	53.2	87.9

Linearity

Sample	Dilution	Measured Conc. (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
	None	28.86	28.86	
	1:2	13.85	14.43	96.0
1	1:4	7.31	7.22	101.3
	1:8	3.78	3.61	104.9
	1:16	1.85	1.80	102.6
	None	56.36	56.36	
	1:2	26.77	28.18	95.0
2	1:4	13.29	14.09	94.3
	1:8	6.75	7.05	95.8
	1:16	3.56	3.52	101.1

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Haemoglobin (up to 2 mg/mL), Bilirubin (up to 0.25 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of IGF-1 in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact us.

Therapeutic Consequences
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point "Reliability of Results". Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point "Therapeutic Consequences". are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!