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Instructions for use

IGF-1 ELISA

REF ME E-0500R

RUO

For research use only – Not for use in diagnostic procedures

IGF-1 ELISA

1 INTRODUCTION

1.1 Intended Use

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

2 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.
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 sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research 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 to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the 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 directly from the manufacturer.

4 REAGENTS

4.1 Reagents provided

ME E-0519 HCL ***0.2 M HCl*** **-** ready to use

Volume: 2 x 3 ml
for sample acidification

Hazards identification:

H290 May be corrosive to metals.

ME E-0587 NEUTR-SOLN ***Neutralization Buffer*** **-** ready to use

Volume: 1 x 3 ml
for neutralization of samples

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

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

Standards and **Controls** - ready to use

Cat. no.	Component	Standard	Concentration	Volume / Vial
ME E-0501	STANDARD A	Standard A	0 ng/ml	1 ml
ME E-0502	STANDARD B	Standard B	10 ng/ml	1 ml
ME E-0503	STANDARD C	Standard C	50 ng/ml	1 ml
ME E-0504	STANDARD D	Standard D	150 ng/ml	1 ml
ME E-0505	STANDARD E	Standard E	300 ng/ml	1 ml
ME E-0506	STANDARD F	Standard F	600 ng/ml	1 ml
ME E-0551	CONTROL 1	Control Low	For control values and ranges please refer to vial label or QC-Report.	1 ml
ME E-0552	CONTROL 2	Control High		1 ml

Conversion: 1 ng/ml x 0.13 = nmol/l

Content: Contain non-mercury preservative
The standards are calibrated against the International Reference Reagent for IGF-1, NIBSC code: 02/254.

ME E-0540 CONJUGATE ***Enzyme Conjugate*** - ready to use

Content: biotinylated IGF-1
contains non-mercury preservative.

Volume: 1 x 14 ml

ME E-0515 ENZYME ***Enzyme Complex*** - ready to use

Content: Streptavidin HRP Complex
contains non-mercury preservative

Volume: 1 x 20 ml

FR E-0055 SUBSTRATE ***Substrate Solution*** - ready to use

Content: Tetramethylbenzidine (TMB)

Volume: 1 x 14 ml

FR E-0080 STOP-SOLN ***Stop Solution*** - ready to use

Content: contains 0.5 M H_2SO_4

Volume: 1 x 14 ml

Avoid contact with the stop solution. It may cause skin irritations and burns.

Hazards identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

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

Volume: 1 x 30 ml

see „Preparation of Reagents“.

Note: Additional *Standard A* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 nm, with reference wavelength at 620 nm - 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled 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

4.3 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 8 weeks if stored as described above.

4.4 Reagent 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 distilled water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 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.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the 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.

5 SPECIMEN COLLECTION AND PREPARATION

Serum can be used in this assay.
NOTE: In plasma significantly reduced values were observed.

Note: Samples containing sodium azide should not be used in the assay.
In general it should be avoided to use haemolytic, icteric or lipaemic specimens. For further information refer to chapter “*Interfering Substances*”.

5.1 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. Donors receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying
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.

5.3 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 A and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:
a) dilution 1:2: 50 µl Serum + 50 µl Standard A (mix thoroughly)
b) dilution 1:10: 10 µl Serum + 90 µl Standard A (mix thoroughly)

6 ASSAY PROCEDURE

6.1 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.

6.2 Acidification and Neutralization of Samples, Standards and Controls

1.	Pipette **50 µl Sample, Standard and 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 **50 µl 0.2 M HCl**.
3.	Mix and incubate for 30 minutes.
4.	For Neutralization add **10 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 the test procedure in chapter 6.3.

6.3 Test Procedure

Each run must include a standard curve

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **20 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 **30 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 **15 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 nm (reading) and at 620 - 630 nm (background subtraction, recommended)** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and 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 Instructions for Use have been calculated automatically using a 4 Parameter curve fit (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods). 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.

6.4.1 Example of Typical Standard Curve

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

Standard	**Optical Units (450 nm)**
Standard A (0 ng/ml)	2.01
Standard B (10 ng/ml)	1.76
Standard C (50 ng/ml)	1.21
Standard D (150 ng/ml)	0.64
Standard E (300 ng/ml)	0.41
Standard F (600 ng/ml)	0.23

7 EXPECTED NORMAL VALUES

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

In a study conducted with apparently normal healthy subjects, using the IGF-1 ELISA the following values are observed:

Age (years)	n	Mean (ng/ml)	2.5th Percentile (ng/ml)	50th Percentile (ng/ml)	97.5th Percentile (ng/ml)	Min. Value (ng/ml)	Max. Value (ng/ml)
Newborn	55	101	65	94	172	65	184
1	10	75	45	77	115	44	119
2	10	91	67	92	120	64	126
3	8	143	103	133	204	103	206
4	11	121	71	119	195	65	208
5	10	165	130	160	232	126	248
6	11	162	80	170	231	62	236
7	10	171	102	169	236	95	242
8	12	200	156	199	249	150	251
9	13	191	61	207	270	44	275
10	11	227	132	237	273	108	278
11	13	227	139	246	308	136	315
12	9	284	174	279	372	158	375
13	3	295	203	265	411	200	419
14	6	269	153	255	416	151	424
15	11	269	119	303	375	111	386
16	9	229	106	228	379	88	407
17	6	251	175	243	349	174	355
18	4	244	186	222	338	184	346
19	5	256	168	246	387	163	402
20	3	317	246	346	363	240	363
21 - 25	23	191	97	175	304	92	304
26 - 30	7	211	137	218	278	133	284
31 - 35	8	184	120	193	229	115	229
36 - 40	5	232	190	220	294	188	300
41 - 45	14	154	107	156	216	105	228
46 - 50	8	150	86	164	196	84	200
51 - 55	17	148	102	140	212	100	214
56 - 60	18	151	96	141	217	91	218
61 - 65	11	130	81	124	216	79	217
66 - 70	10	119	80	117	176	79	183
71 - 75	15	124	57	124	199	46	212
76 - 80	14	122	74	115	192	73	205

8 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 Report 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 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 or the manufacturer directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 9.75 ng/ml – 600 ng/ml.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Compound	% Cross reactivity
IGF-1	100
IGF-2	1.02
Insulin	3.3

9.3 Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard A and was found to be 9.75 ng/ml.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	20	89.34	7.39
2	20	227.39	6.93
3	20	390.82	6.39

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	40	14.50	14.84
2	40	66.26	10.34
3	40	125.30	12.63

9.5 Recovery

Samples have been spiked by adding IGF-1 solutions with known concentrations in a 1:1 ratio.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous IGF-1 + added IGF-1) / 2; because of a 1:2 dilution of serum with spike material).

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Concentration (ng/ml)		91.60	158.08	170.47	173.55	190.48
Average Recovery (%)		103.5	102.0	95.8	109.2	95.9
Range of Recovery (%)	from	96.8	96.6	86.1	98.8	87.5
	to	106.6	106.0	114.9	126.4	101.2

9.6 Linearity

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Concentration (ng/ml)		141.55	199.55	201.36	250.29	301.28
Average Recovery (%)		94.4	94.3	97.7	95.1	97.4
Range of Recovery (%)	from	88.2	85.4	87.5	89.8	91.4
	to	101.3	107.3	112.7	101.8	105.6

10 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.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.25 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.

A biotin concentration of up to 1200 ng/ml in a sample has no influence on the assay results.

10.2 Drug Interferences

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

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

12 REFERENCES / LITERATURE

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!