

Instructions for use

IGF-1 ELISA

Please use only the valid version of the Instructions for Use provided with the kit

REF**ME E-0500R**

96

RUO

For research
use only –
Not for use
in diagnostic
procedures

IGF-1 ELISA

1 INTENDED USE

The **IGF-1 ELISA** is a manual enzyme immunoassay for the quantitative measurement of Insulin-like Growth Factor 1 (IGF-1) in human 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.

During the first incubation, IGF-1 in the added pretreated sample competes with an IGF-1-biotin conjugate (Enzyme Conjugate) for binding to the coated antibody. After incubation, the microtiter plate is washed to stop the competition reaction. In the following incubation the bound biotin molecules are detected with streptavidin peroxidase (Enzyme Complex).

After a second washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured.

The intensity of color is inversely proportional to the concentration of the analyte in the sample.

A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

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 H₂SO₄. 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 MATERIALS

4.1 Materials 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 **W 96** **Microtiterwells**
 Content: 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 1	For control values and ranges please refer to vial label or QC-Report.	1 ml
ME E-0552	CONTROL 2	Control 2		1 ml

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

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

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 H₂SO₄

Volume: 1 x 14 ml

Avoid contact with the Stop Solution. It may cause skin irritations and burns.

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

Volume: 1 x 30 ml

See „Preparation of Reagents“.

Instructions for Use

Certificate of analysis (CoA)

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 distilled 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 1 week 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 SAMPLE 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 hemolytic, icteric or lipaemic specimens. For further information refer to chapter "*Interfering Substances*".

5.1 Sample 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 Sample Storage and Preparation

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

Samples held for a longer time (up to 14 months) 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 sample is found to contain more than the highest standard, the samples 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 <u>not necessary</u> . <u>Immediately (within 10 minutes)</u> 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 <u>acidified and neutralized</u> Standard, Control , and samples <u>with new disposable tips</u> 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 <i>Wash Solution</i> (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 <i>Wash Solution</i> (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 the solution in 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 <i>Stop Solution</i> .

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 REFERENCE VALUES

It is strongly recommended that each laboratory should determine its own reference 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)	2.5 th Percentile (ng/ml)	50 th Percentile (ng/ml)	97.5 th 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	129	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

The following values were found for suitable subjects differentiated by sex and age:

Females

Age (years)	n	Mean (ng/ml)	Median (ng/ml)	5 th – 95 th Percentile (ng/ml)	1 st – 99 th Percentile (ng/ml)	Range (min. – max.) (ng/ml)
1 – 10	21	194.88	200.69	104.88 – 277.94	83.83 – 326.67	78.57 – 338.85
11 – 20	6	303.63	301.30	217.87 – 392.02	163.34 – 399.63	208.65 – 401.53
21 – 40	38	211.05	209.23	109.34 – 303.44	95.56 – 330.23	91.74 – 345.75
41 – 60	22	161.83	159.31	111.29 – 217.39	106.29 – 225.95	105.03 – 228.12
61 – 80	20	128.96	126.27	78.73 – 212.21	77.66 – 216.35	77.39 – 217.38

Males

Age (years)	n	Mean (ng/ml)	Median (ng/ml)	5 th – 95 th Percentile (ng/ml)	1 st – 99 th Percentile (ng/ml)	Range (min. – max.) (ng/ml)
1 – 10	39	167.22	165.09	54.82 – 293.30	49.31 – 328.30	46.14 – 340.02
11 – 20	4	200.08	198.61	165.88 – 236.34	163.34 – 239.58	162.71 – 240.39
21 – 40	6	136.83	133.57	108.28 – 167.08	95.84 – 171.41	101.28 – 172.50
41 – 60	36	148.08	140.25	97.25 – 214.16	88.02 – 273.90	83.77 – 305.37
61 – 80	30	120.85	116.52	74.64 – 191.55	53.59 – 210.48	45.62 – 212.77

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard 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 analyzing 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 Specificity of Antibodies (Cross Reactivity)

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

Substance	Conc. Range of Spiked Substance ng/ml	Mean Cross-Reactivity %
IGF-1	–	100.0
Insulin	0.0043 – 4.33	3.3
IGF-2	300.00 – 1200.00	1.0

9.2 Detection Capability

Limit of Blank (LoB)	0.808 ng/ml
Limit of Detection (LoD)	4.081 ng/ml
Limit of Quantification (LoQ)	10.264 ng/ml
Measuring range	4.081 ng/ml – 600.0 ng/ml
Linear range	8.847 ng/ml – 600.0 ng/ml

9.3 Repeatability and Reproducibility

9.3.1 Repeatability (within-run precision)

The repeatability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	20	89.34	7.4
2	20	227.39	6.9
3	20	390.82	6.4

9.3.2 Reproducibility (between-run precision)

The reproducibility (between-run) is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	40	14.50	14.8
2	40	66.26	10.3
3	40	125.30	12.6

9.4 Reproducibility (between-lot precision)

The reproducibility (between-lot) is shown below:

Sample	Mean Conc. (ng/ml) Lot 1	Mean Conc. (ng/ml) Lot 2	Mean Conc. (ng/ml) Lot 3	Mean Conc. (ng/ml)	Between-Lot CV (%)	n
1 (Serum)	74.93	78.98	80.14	78.02	3.5	18
2 (Serum)	94.83	90.82	91.12	92.26	2.4	18
3 (Serum)	115.87	118.94	119.14	117.98	1.6	18
4 (Serum)	155.78	163.93	138.83	152.85	8.4	18

9.5 Spiked 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
Concentration (ng/ml)		91.60	158.08	170.47	190.48
Average Recovery (%)		103.5	102.0	95.8	95.9
Range of Recovery (%)	from	96.8	96.6	86.1	87.5
	to	106.6	106.0	114.9	101.2

9.6 Linearity of Dilution

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (ng/ml)		141.55	199.55	201.36	250.29	301.28	576.6
Average Recovery (%)		94.4	94.3	97.7	95.1	97.4	108.2
Range of Recovery (%)	from	88.2	85.4	87.5	89.8	91.4	91.6
	to	101.3	107.3	112.7	101.8	105.6	108.2

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

In general, hemolytic, icteric or lipemic samples should be avoided.

Unconjugated bilirubin (up to 0.3 mg/ml), conjugated bilirubin (up to 0.1 mg/ml), Hemoglobin (up to 4 mg/ml), triglyceride (up to 7.5 mg/ml), cholesterol (up to 4 mg/ml), ethanol (up to 4 mg/ml), and glucose (up to 12.5 mg/ml) have no influence on the assay results.

10.2 Biotin Interference

Biotin interference is not detected up to a concentration of 1200 ng/ml for this assay.

10.3 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence on the measurement of IGF-1 600 in a sample.

10.4 High-Dose-Hook Effect

A high dose Hook effect was not detected for this test.

11 LEGAL ASPECTS

11.1 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 the manufacturer.

11.2 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 11.2 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.

12 REFERENCES / LITERATURE

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture				For research use only!