



IGF-1-ELISA

Enzyme linked immunosorbent assay for the determination of Insulin like Growth Factor -1 in human serum- code : BL-49-E
IN VITRO DIAGNOSTIC USE



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1. Intended use

For **IN VITRO** determination of serum Insulin like growth factor-1 (IGF-1) levels. Insulin like growth factor-I(IGF-I) also called Somatomedin-C is a low molecular weight peptide (PM : 7649; 70 aa) which promotes cellular mitosis and differentiation in a variety of tissues.

The liver is the main source of circulating IGF-I and its synthesis is regulated by GH. Several other tissues produce IGF-I and these productions are regulated by GH or others modulators.

Contrary to the ultradian rhythm of GH secretion, the IGF-I secretion pattern presents negligible nyctemeral variations.

The majority of circulating IGF-I is bound to plasma IGF binding proteins (IGFBPs), particularly IGFBP-3. Less than 1% IGF-I is free. Circulating IGFBP-3 is directly correlated with the rate of GH secretion.

In humans, serum IGF-I levels are low during foetal and neonatal life, increase gradually during puberty, peaking at Tanner stages 3-4, and show a decline similar to GH with ageing. In females at each age, average IGF-I plasma levels are slightly higher than in males.

There is a significant correlation between IGF-I values and plasma sex steroid concentrations. Sex steroids influence IGF-I levels via an increase in GH secretion. In addition, IGF-I levels are dependent on both caloric intake and protein content of the diet.

In nanism due to congenital GH deficiency or GH resistance (Laron dwarf), as well as in later forms of GH deficiency one observes a decrease in circulating IGF-I. This also causes a decrease in serum levels of IGFBP-3 (GH dependent).

Elevations of serum GH levels produce an increase of IGF-I, insulin and IGFBP-3, and a decrease of IGFBP-1 and -2. This is typically observed under GH administration and in acromegaly.

In obesity, serum levels of insulin and IGF-I are increased, but GH level is decreased. This seems to be due to feedback inhibition by excess of IGF-I.

Summary :

| Parameter Status | GH level | Insulin level | IGF-I level | IGFBP-3 level | GHPB level |
|------------------|----------|---------------|-------------|---------------|------------|
| Normal | N | N | N | N | N |
| ↓ GH | ↓ | ↓ | ↓ | ↓ | ↓ |
| ↑ GH | ↑ | ↑ | ↑ | ↑ | ↑ |
| Obesity | ↓ | ↑ | N or ↑ | N | N or ↓ |
| Malnutrition | ↑ | ↓ | ↓ | ↓ | ↓ |
| Laron dwarf | ↑ | ↓ | ↓ | ↓ | ↓↓ |

GH BP : Growth Hormone Binding Proteins
N : NORMAL.

2. Principle of the method

As IGFBP-3 interferes in the determination of IGF-1 level, it is essential to include an extraction step in which IGF-1 is separated from its binding protein.

The ELISA technique uses antibodies with high affinity and specificity for two different epitopes on IGF-1. A first monoclonal anti-IGF-1 antibody bound to a polystyrene well will capture the IGF-1 of the sample in the presence of a second alkaline phosphatase conjugated monoclonal anti-IGF-1 antibody.

Following the incubation and the one step formation of the solid phase-IGF-1-conjugated monoclonal antibody sandwich, the well is washed to remove excess of unbound conjugated antibody. Then the chromogen/substrate is added, which turns from clear to yellow proportionally to IGF-1 concentration in the patient sample. The intensity of the yellow color is measured using a spectrophotometer with a 405 nm filter.

Patient sample concentrations are read from a calibration curve.

3. Warnings and precautions

For **in vitro diagnostic use**

It must be handled by specialized staff.

Good laboratory and safety practices are advisable.

Warning : Some components contain sodium azide (<1g/l). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide building up.

Warning : This kit contains human origin materials which are tested negative for HBs antigen, anti- HIV 1 and 2 and anti-HCV antibodies. Animal origin materials are also used in this kit, these are provided with sanitary certificate. However, no known test can guarantee that such material does not contain any of these infectious agents or other infectious agents. These products must be considered as potentially infectious and handled with care.

4. Reagents, preparation and storage

All reagents are ready for use, except the washing solution, calibrators (0-6) and controls (1-2).

Stored at 2-8 °C, the material can be used up to the expiration date printed on each label. The diluted washing solution can be stored at 2-8 °C or 18-25 °C.

Before use, reconstitute the content of the calibrators (0-6) and controls with 0.5 ml of deionized water. Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing. If not used immediately after reconstitution, store aliquots at -20 °C for up to 4 weeks.

After use, close all reagents vials and bottles and replace these at 2-8 °C or -20 °C. Store the unused strips/wells with the dessicant sachet in the provided minigrig bag at 2-8 °C. Do not forget to reseal the bag.

- 96 breakable wells polystyrene microplate coated with mouse anti-IGF-1 monoclonal antibody. Systematically allow the microwells microplate to reach room temperature before opening the bag. Single use strips/wells.
- | | |
|----|----|
| Ab | AP |
|----|----|

 1 bottle (11 ml, red) alkaline phosphatase conjugated mouse anti-IGF-1 monoclonal antibody diluted in buffer containing preservative (NaN₃ < 1 g/l) and a red dye.
- | | |
|-----|---|
| CAL | N |
|-----|---|

 7 vials recombinant human IGF-1 lyophilized in buffer containing preservatives (NaN₃ < 1g/l). The calibrators are calibrated against the WHO 1st IGF-1 International Standard (coded 87/518). The concentrations expressed in ng/ml of the calibrators are printed on the vial labels.
- | | |
|---------|---|
| CONTROL | N |
|---------|---|

 2 vials recombinant human IGF-1 lyophilized in buffer containing preservatives (NaN₃ < 1g/l). The controls have to be assayed with the patient samples and the results compared with those printed on the vials
- | | |
|------|------|
| EXTR | SOLN |
|------|------|

 1 bottle (45 ml) of extraction solution : ethanol acid solution (87.5% ethanol + 12.5% HCl 2.0 N).
- | | |
|-------|------|
| NEUTR | SOLN |
|-------|------|

 1 vial (6 ml) of a neutralizing buffer.
- | | |
|-----|-----|
| DIL | SPE |
|-----|-----|

 1 vial (10 ml) of sample diluent containing preservatives (NaN₃ < 1 g/l).
- | | |
|-----|------|
| SUB | PNPP |
|-----|------|

 2 bottles (10 ml/bottle) PNPP diluted in buffer containing preservatives.
- | | |
|------|------|
| STOP | SOLN |
|------|------|

 1 bottle (40 ml) diluted buffer containing preservatives (NaN₃ < 1 g/l).
- | | | |
|------|------|------|
| WASH | SOLN | CONC |
|------|------|------|

 1 bottle (60 ml) concentrated buffer solution containing preservatives (NaN₃ < 1 g/l). Pour the solution in 1140 ml of distilled water and homogenize.

5. Material required but not provided

- Test tubes for the dilutions and a tube holder
- Vortex mixer
- Manual or automated precision micropipettes with single use tips for dispensing samples or reagents without cross-contamination.
- Multichannel micropipette or repeating dispenser (Eppendorf type)
- Vacuum pump connected through a trap for aspiration
- 96-well microplate reader with a 405 nm filter
- Semi-logarithmic paper (or software package)
- Microplate washer (facultative).
- Microcentrifuge tubes (for example: SARSTEDT n° 72.690.550).
- Microcentrifuge or a centrifuge.
- Reciprocating or orbital shaker (200 - 800 rpm).

6. Methodology

6.1. Collection and handling of serum samples

The blood sample may be collected into a dry tube. EDTA must be avoided.

The serum when separated from the red blood cells, may be assayed immediately, within 24 hours if stored at 2-8 °C, or after periods up to several months if stored at -20 °C.

Repeating freezing and thawing must be avoided.

Do not mix reagents of different lots.

Bring the different components of the kit to room temperature prior to use. Perform the assay in duplicates. Calibrators, controls and samples must be assayed at the same time. Follow strictly the different steps of the procedure and use interchangeable tips.

6.2. Serum extraction procedure

Do not extract the calibrators and controls.

6.2.1. Procedure for microcentrifuge tubes

- Label 1 polypropylene microcentrifuge tube for each sample (extraction tube). Label 1 tube for each sample (neutralization tube).
- Add 50 µl of each sample into the corresponding microcentrifuge tubes.
- Add 400 µl of extraction solution (EXTR SOLN) to the sample. Close the tubes, vortex and incubate 30 minutes at room temperature.
- Centrifuge at ≥ 10,000 rpm for 2 minutes at 4 °C.
- Without disturbing the pellet, transfer 100 µl of clear supernatant into the neutralization tube.
- Add 50 µl of neutralizing solution (NEUTR SOLN) and vortex gently.
- Add 100 µl of sample diluent (DIL SPE). Vortex gently the solution that should be used in 6.3.

6.2.2. Procedure for other tubes.

All the steps are the same than 6.2.1. except point 1 and 4 :

- Label 2 tubes for each sample - (one for extraction, and the second one for neutralization of the sample) ;
- Centrifuge at $\geq 5,000$ rpm for 30 minutes at 4°C. With a time adjustment it is possible to centrifuge with lower speed.

6.3. Assay Procedure

Select the number of coated wells for IGF-1 assays. Replace unrequired wells/strips in the MINIGRIP bag along with the dessicant bag and seal tightly.

1. Calibrators

Dispense 25 μ l of each calibrator into the appropriate wells.

2. Samples and controls

Dispense 25 μ l extracted sample or controls into appropriate wells.

- Add 100 μ l of conjugate into each well.
- Incubate for 90 minutes at room temperature (25°C) on a reciprocating or orbital shaker (200-800 rpm).
- Flick out the contents of the wells over a basin containing bleaching water or aspirate with an automated plate washer.
- Wash the wells three times with an automated system set to 250 μ l per well, or by adding 250 μ l to each well, flicking out over a basin and blotting the wells on absorbent paper to remove any residual liquid after each washing.
- Dispense 100 μ l of chromogen/substrate (SUBS PNPP) solution into each well, ensuring that it is initially pale coloured.
- Incubate for 30 minutes at room temperature (25°C).
- Stop the reaction by adding 100 μ l of stop solution (STOP SOLN) to each well.
- Place the plate on a flat surface, swirl gently to mix contents or use the option « mixing » if your reader has one.
- Measure the absorbance at 405 nm on a 96 well microplate reader.

6.4. Data Processing

Draw a calibration curve on semilogarithmic paper by plotting mean absorbance (linear scale) obtained for each standard versus its respective concentration expressed in ng/ml (logarithmic scale). IGF-1 concentrations in sample may be read directly from the appropriate standard curve.

If a computer is used to calculate the results, the data can be fitted to the appropriate equation : POINT TO POINT, sigmoid, polynomial (Spline, Cubic),

Since the dilution factor has already been considered in the standard curve calibration, the IGF-1 concentrations need no conversion. Any sample values above the standard range should be diluted and retested.

6.5. Example of typical assays

| | Contents (ng/ml) | ABS 405 nm 1° duplicate | ABS 405 nm 2° duplicate | Mean ABS | IGF-1 (ng/ml) |
|-----------|------------------|----------------------------|----------------------------|-------------|------------------|
| CAL 0 | 0 | 0.071 | 0.071 | 0.071 | |
| CAL 1 | 45 | 0.184 | 0.175 | 0.180 | |
| CAL 2 | 77 | 0.301 | 0.300 | 0.301 | |
| CAL 3 | 133 | 0.543 | 0.499 | 0.521 | |
| CAL 4 | 270 | 0.887 | 1.006 | 0.947 | |
| CAL 5 | 570 | 2.072 | 2.165 | 2.199 | |
| CAL 6 | 1330 | 2.721 | 2.776 | 2.749 | |
| Control 1 | 65.5 - 107.5 | 0.308 | 0.262 | 0.285 | 88.9 |
| Control 2 | 217.7 - 487.9 | 1.280 | 1.561 | 1.421 | 358.5 |
| Sample 1 | | 1.026 | 1.098 | 1.062 | 273.2 |
| Sample 2 | | 1.229 | 1.200 | 1.215 | 308.1 |
| Sample 3 | | 0.431 | 0.425 | 0.428 | 130.3 |
| Sample 4 | | 1.086 | 1.266 | 1.173 | 299.0 |

Examples of typical assay performed at controlled temperature of 25°C.
Do not use for calculations

7. Expected normal values

It is recommended that each laboratory establishes its own reference values.

SEE TABLE AND CURVES ATTACHED.

8. Limitation of the procedure

- The results obtained from this or any other diagnostic kit should be used and interpreted only in the context of an overall clinical picture.
- Do not use strongly lipemic, haemolyzed, icteric or turbid specimens
- Special care is needed to prevent contamination of the substrate by the conjugate. The substrate should be uncoloured or pale yellow, a franc yellow indicates that the reagent has been contaminated and must be discarded. Substrate degradation is increased at temperatures above 25°C.
- The well washing procedure is critical for the successful performance of the test.
- Do not use EDTA tubes to collect blood samples.

9. Quality control

Use the controls provided for each assay.

If, in normal using conditions, the controls are out the acceptable ranges, the sample results can't be validated. Please contact the manufacturer.

10. Performance characteristics

10.1. Specificity

The relative percent of cross-reactivity of IGF-1 and other related compound was evaluated in this assay. The cross-reactivity was determined as the ratio of the apparent increase in IGF-1 level (ng/ml) to the concentration of the potentially cross reacting compound (ng/ml).

| COMPOUNDS | CROSS REACTIVITY (%) |
|-----------|----------------------|
| IGF - II | < 0.01 |
| INSULIN | < 0.1 |
| GH | < 0.1 |

10.2. Sensitivity

10.2.1. Analytical sensitivity

The minimum detectable concentration of IGF-1 has been assayed at 4.9 ng/ml and corresponds to the concentration given by two standard deviations above the mean mOD of 25 replicates determinations of the zero standard.

10.2.2. Functional sensitivity

The functional sensitivity has been assayed at 8 ng/ml and corresponds to a between assay variation of 20 % (n=20).

10.3. Imprecision

| | Repeatability | Within Assay Variation |
|--------|--------------------|------------------------|
| | Mean value (ng/ml) | % CV (20 replicates) |
| Pool 1 | 153 | 7.8 |
| Pool 2 | 305 | 6.6 |
| Pool 3 | 375 | 9.7 |

| | Reproducibility | Between Assay Variation |
|--------|--------------------|-------------------------|
| | Mean value (ng/ml) | % CV (20 replicates) |
| Pool 1 | 93 | 11.3 |
| Pool 2 | 144 | 13.3 |
| Pool 3 | 315 | 13.7 |

10.4. Recovery Test

When known concentrations of IGF-1 are added to sera of known IGF-1 concentrations, a satisfactory correlation between expected (endogenous + added hormone) and assayed IGF-1 is obtained.

| Sample + x ng/ml | Expected Conc. ng/ml | Assayed Conc. ng/ml | % Recovery |
|---------------------|-------------------------|------------------------|------------|
| 69 + 388 | 457 | 463 | 101.5 |
| 69 + 194 | 263 | 282 | 109.8 |
| 69 + 97 | 166 | 171 | 105.1 |

10.5. Dilution test

The dilution test indicates that there is immunological identity between the IGF-1 present in serum and the IGF-1 used to calibrate the standard curve.

| Dilution Factor | Expected Conc. ng/ml | Assayed Conc. ng/ml |
|-----------------|----------------------|---------------------|
| 1 | - | 990 |
| 1/2 | 495 | 383 |
| 1/4 | 248 | 264 |
| 1/8 | 124 | 90 |
| 1/16 | 62 | 67 |

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