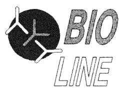

IGFBP-3-ELISA

Enzyme linked immunosorbent assay for the determination of Insulin like Growth Factor Binding Protein-3 in human serum - BL-50-E

IN VITRO DIAGNOSTIC USE

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium Tel: +32-2-736.62.18.Fax: +32-2-742.13.15.

1. Intended use

For **IN VITRO** determination of serum IGFBP-3 levels.

IGFBP-3 is found in human serum and has two forms of approximate molecular mass 40 and 43 kDa.

IGFBP-3 binds IGF-1 and IGF-2 with similar high affinity. In the blood stream, approximately 90 % of IGFBP-3 is found in a ternary complex of 140 kDa. This complex comprises an acid-labile subunit (α-subunit), IGFBP-3 (β-subunit) and IGF-1 or IGF-2. This binding prolongs the metabolic half-life of IGF-1 in the circulation and serves as a metabolic reservoir of IGF-1.

Due to stable circadian levels, a single IGFBP-3 measurement proved to be sufficient in contrast to GH measurement. Serum levels of IGFBP-3 are low at birth, rise rapidly during the first weeks of life, reach a peak at the time of puberty and fall during adult life.

Clinical studies have demonstrated the important carrier function of IGFBP-3. When GH and IGF-1 production are increased, as in acromegaly, increases in circulating IGFBP-3 and α-subunit are seen. As the IGF-2 level does not increase, the excess of IGF-binding sites are all filled by IGF-1. Conversely, when GH and IGF-1 production are decreased, as in GH-deficiency, IGFBP-3 and α-subunit levels fall.

In patients with GH deficiency (GHD), the response of IGFBP-3 to GH administration is slow (maximum after 4 days). In contrast, the response to IGF-1 administration is considerably faster (maximum after 4 hours) indicating that IGFBP-3 may be regulated by IGF-1 rather than GH.

The determination of the blood level of IGFBP-3 has been proposed as the best indicator of GHD during the 5 first years of life because of the poor discrimination value of IGF-1 levels. In fact, during this period, normal levels of IGF-1 are very low.

It can be concluded that :

1. A single IGFBP-3 determination is an excellent screening parameter for GHD.
2. IGFBP-3 is a good parameter for monitoring the therapeutic efficacy in both GHD an acromegaly.

<u>Summary</u> :

Parameter Statuts	GH concentration	Insulin concentration	IGF-1 concentration	IGFBP-3 concentration	GH-BP concentration
Normal	N	N	N	N	N
↓ GH (GHD)	↓	↓	↓	↓	↓
↑ GH (Acromegaly, Gigantism)	↑	↑	↑	↑	↑
Obesity	↓	↑	N or ↑	N	N or ↓
Malnutrition	↑	↓	↓	↓	↓
Laron dwarf	↑	↓	↓	↓	↓↓

GH BP : Growth Hormone Binding Proteins
N : NORMAL.

2. Principle of the method

The ELISA technique uses antibodies with high affinity and specificity for two different epitopes on IGFBP-3. A first monoclonal anti-IGFBP-3 antibody bound to a polystyrene well will capture the IGFBP-3 of the sample in the presence of a second alkaline phosphatase conjugated monoclonal anti-IGFBP-3 antibody.

Following the incubation and the one step formation of the solid phase-IGFBP-3-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 IGFBP-3 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.

<u>Warning</u> : 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.

<u>Warning</u> : 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 (0-5) and controls.

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 (0-5) and controls with 1 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 8 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 minigrip bag at 2-8°C. Do not forget to reseal the bag.

4.1 [microplate] 96 breakable wells polystyrene microplate coated with mouse anti IGFBP-3 monoclonal antibody. Systematically allow the microwells microplate to reach room temperature before opening the bag. Single use strips/wells.

4.2 [Ab | AP] 1 bottle (11 ml, red) of alkaline phosphatase conjugated mouse IGFBP-3 monoclonal antibody diluted in buffer containing preservative (NaN_3 < 1 g/l) and a red dye.

4.3 [CAL | N] 6 vials of affinity purified human IGFBP-3 lyophilized in buffer containing preservatives (NaN_3 < 1g/l). The calibrators are standardized against the NIBSC/WHO recombinant IGFBP-3, reference reagent coded 93/560. The concentrations expressed in ng/ml of the calibrators are printed on the vial labels.

4.4 [CONTROL | N] 2 vials of affinity purified human IGFBP-3 lyophilized in buffer containing preservatives (NaN_3 < 1g/l). The controls have to be assayed with the patient samples and the results compared with those printed on the vials

4.5 [DIL | SPE] 1 vial (100 ml) of diluent buffer containing preservatives (NaN_3 < 1 g/l).

4.6 [SUB | PNPP] 2 bottles (10 ml/bottle) PNPP diluted in buffer containing preservatives.

4.7 [STOP | SOLN] 1 bottle (40 ml) diluted buffer containing preservatives (NaN_3 < 1 g/l)..

4.8 [WASH | SOLN | CONC] 1 bottle (60 ml) concentrated buffer solution containing preservatives (NaN_3 < 1 g/l). Poor 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).
- Reciprocating or orbital shaker (200 - 800 rpm).

6. Methodology

6.1. <u>Collection and handling of serum samples</u>

The blood sample may be collected either into a dry tube. EDTA and NaF 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. <u>Serum dilution</u>

Each sample must be diluted 1/101

10 µl sample + 1.0 ml sample diluent (DIL SPE)

Homogenize each dilution

Calibrators and controls are already prediluted and must be used without any further dilution

6.3. Assay Procedure

Select the number of coated wells for IGFBP-3 assays. Replace unrequired wells/strips in the MINIGRIP bag along with the dessicant bag and seal tightly.

1. Calibrators
   Dispense 100 µl of each calibrator into the appropriate wells.
2. Samples and controls
   Dispense 100 µl diluted sample or control into appropriate wells.
3. Add 100 µl of conjugate into each well.
4. Incubate for 90 minutes at room temperature (25°C) on a reciprocating or orbital shaker (200-800 rpm).
5. Flick out the contents of the wells over a basin containing bleaching water or aspirate with an automated plate washer.
6. Wash the wells three times with an automated system set to 250 µl per well, or by adding 250 µl to each well, flicking out over a basin and blotting the wells on absorbent paper to remove any residual liquid after each washing.
7. Dispense 100 µl of chromogen/substrate (SUB PNPP) solution into each well, ensuring that it is initially pale coloured.
8. Incubate for 30 minutes at room temperature (25°C).
9. Stop the reaction by adding 100 µl of stop solution (STOP SOLN) to each well.
10. Place the plate on a flat surface, swirl gently to mix contents or use the option « mixing » if your reader has one.
11. 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). IGFBP-3 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 , sigmoïd, polynomial (Spline, Cubic), ...

☞ Since the dilution factor has already been considered in the standard curve calibration, the IGFBP-3 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	IGFBP-3 (ng/ml)
CAL0	0	0.068	0.072	0.070	
CAL1	361	0.232	0.236	0.234	
CAL2	773	0.442	0.420	0.431	
CAL3	2107	1.044	1.016	1.030	
CAL4	4058	1.727	1.753	1.740	
CAL5	6902	2.349	2.419	2.384	
Control 1	454-676	0.319	0.339	0.329	559
Control 2	1422-2214	0.872	0.884	0.878	1752
Sample 1		0.432	0.436	0.434	779
Sample 2		0.687	0.693	0.690	1330
Sample 3		1.448	1.394	1.421	3104
Sample 4		1.678	1.628	1.653	3780

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

8.1. The results obtained from this or any other diagnostic kit should be used and interpreted only in the context of an overall clinical picture.

8.2. Do not use strongly lipemic, haemolyzed, icteric or turbid specimens

8.3. 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.

8.4. The well washing procedure is critical for the successful performance of the test.

8.5. Do not use EDTA or NaF 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 IGFBP-3 and other related compound was evaluated in this assay. The cross-reactivity was determined as the ratio of the apparent increase in IGFBP-3 level (ng/ml) to the concentration of the potentially cross reacting compound (ng/ml).

COMPOUNDS	CROSS REACTIVITY (%)
IGF - I	< 0.001
IGFBP-1	< 0.001
IGFBP-2	< 0.001

10.2. Sensitivity

10.2.1. Analytical sensitivity

The minimum detectable concentration of IGFBP-3 has been assayed at 10.5 ng/ml and corresponds to the concentration given by two standard deviations above the mean cpm of 25 replicates determinations of the zero standard.

10.2.2. Functional sensitivity

The functional sensitivity has been assayed at 135 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	1020	4.3
Pool 2	2420	6.9
Pool 3	3627	8.3
	Reproductibility	**Between Assay Variation**
	Mean value (ng/ml)	% CV (20 replicates)
Pool 1	337.0	13.7
Pool 2	1684	5.4
Pool 3	3627	8.3

10.4. Recovery Test

When known concentrations of IGFBP-3 are added to sera of known IGFBP-3 concentrations, a satisfactory correlation between expected (endogenous + added hormone) and assayed IGFBP-3 is obtained.

Sample + x ng/ml	Expected Conc. ng/ml	Assayed Conc. ng/ml	% Recovery
1300 + 190	1490	1563	138.4
1300 + 512	1812	1985	133.7
1300 + 1625	2925	3048	107.6
1300 + 3500	4800	4632	95.2

10.5. Dilution test

The dilution test indicates that there is immunological identity between the IGFBP-3 present in serum and the IGFBP-3 used to calibrate the standard curve.

Dilution Factor	Expected Conc. ng/ml	Assayed Conc. ng/ml
1	-	2616
1/2	1308	1423
1/4	654	743
1/8	327	332

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