



IGFBP-3-Irma-CT

Immunoradiometric assay on coated tubes for the determination of Insulin like Growth Factor Binding Protein-3 in human serum



Code: BL-48-CT

IN VITRO DIAGNOSTIC USE

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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 and acromegaly.

Summary :

Parameter Status	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 sensitive IGFBP-3 IRMA is a one step solid phase immunoradiometric assay. A first monoclonal anti-IGFBP-3 antibody bound to a polystyrene tube will capture the IGFBP-3 of the sample in the presence of a second ¹²⁵I labelled monoclonal anti- IGFBP-3 antibody.

Following the incubation and the one step formation of the solid phase-IGFBP-3-labelled monoclonal antibody sandwich, the tube is washed to remove excess of unbound labelled antibody. The radioactivity of the sandwich is directly proportional to the amount of IGFBP-3 present in the sample.

Patient samples 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

This kit contains ¹²⁵I emitting X and ionizing rays.

This radioactive material may be received, acquired, possessed and used only by persons in clinical or hospital laboratories who are authorized by competent authorities and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, transfer, the waste disposal and the people protection are subject to the State and local regulations.

Use impermeable gloves and appropriate protection clothes.

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.


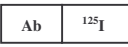


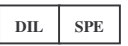

4. Reagents, preparation and storage

All reagents are ready for use, except the washing solution, calibrators (0-5) 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 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 8 weeks.

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

- 4.1.  2 x 50 polystyrene tubes coated with mouse anti- IGFBP-3 monoclonal antibody. Systematically allow the coated tubes to reach room temperature before opening the bag. Single use tubes.
- 4.2.  1 bottle (42 ml, red) of ¹²⁵I-labelled mouse anti IGFBP-3 MAb in buffer with a stabilizer, a preservative (NaN₃ < 1 g/l) and a red dye. Each vial contains a maximum of 370 kBq (10 μCi) of radioactivity at the iodination date.
- 4.3.  6 vials of affinity purified human IGFBP-3 lyophilized in buffer containing preservatives (NaN₃ < 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.  2 vials of affinity purified human IGFBP-3 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
- 4.5.  1 bottle (100 ml) of samples diluent buffer with preservatives (NaN₃ < 1 g/l)
- 4.6.  1 bottle (100 ml) of concentrated buffered solution with preservatives (NaN₃<1g/l). Pour the solution in 900 ml of distilled water and homogenize.

5. Material required but not provided

- bench surfaces protected by absorbent paper to reduce the effects of radioactive spillage.
- waste disposal containers appropriately labelled and designed as suitable for solid or liquid radioactive materials and biological materials.
- manual or automated precision micropipettes with single use tips for dispensing samples or reagents without cross-contamination.
- repeater pipettes (Eppendorf type)
- vacuum pump connected through a trap for aspiration
- reciprocating or orbital shaker (max 350 rpm)
- a calibrated gamma scintillation counter
- appropriate graph paper for plotting the results.

6. Methodology

6.1. Collection and handling of serum samples

The blood sample may be collected into dry tubes.

The serum when separated from the red blood cells may be assayed immediately or 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 dilution

Each sample must be diluted 1/101.

10 µl sample + 1.0 ml sample diluent buffer.

Homogenize each dilution.

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

6.3 Assay procedure

Label the tubes for T ("Total count"), calibrators, controls and samples.

1. Calibrators

Pipette 50 µl of each reconstituted calibrator into the corresponding tubes.

2. Samples and controls

Pipette 50 µl of each diluted sample or controls into the corresponding tubes.

3. Add 400 µl of MAb IGFBP-3 ¹²⁵I tracer (red) to each tube. The "Total count" tubes do not participate in the following steps.

4. Incubate 3 hours at room temperature (20-30 °C) on a reciprocating or orbital shaker (max. 350 rpm)

5. Carefully aspirate or decant the incubation mixture of all tubes.

6. Add 2 ml of washing buffer to each tube. Wait for 1 minute and aspirate or decant carefully.

7. Repeat the washing procedure (step 6)

8. Count the radioactivity fixed in each tube for 1 minute in a gamma scintillation counter.

N.B. If the tubes are decanted, allow them to stand upside-down for a few minutes on absorbent paper; any liquid left in the tubes could modify the fixation and thus impair test quality

6.4 Data processing

Determine the mean count rate for each set of duplicate tubes. Calculate the ratio B/T as follows :

$$B/T (\%) = (\text{Cal or Smp cpm} / \text{T cpm}) \times 100$$

Draw the standard curve on semilogarithmic paper by plotting the ratio B/T % (linear scale) obtained for each calibrator versus its respective concentration expressed in ng/ml (logarithmic scale).

IGFBP-3 concentration in samples may be read directly from the calibration curve.

Since the dilution factor has already been considered in the calibrator curve, IGFBP-3 sample concentration need no conversion.

If a computer is used to calculate the results, the data can be fitted to the appropriate equation : polynomial (spline), 4 parameters, logistics, point to point interpolation.

6.5. Example of a typical assay

	Contents ng/ml	cmp 1st duplicate	cmp 2nd duplicate	Mean count rate	B/T %	IGFBP-3 conc. ng/ml
Total counts	-	124708	124701	124704	-	-
Cal 0	0	535	561	548	0.44	
Cal 1	300	1364	1378	1371	1.09	
Cal 2	840	2890	2926	2908	2.33	
Cal 3	1700	6463	6253	6358	5.98	
Cal 4	4000	17739	17759	17749	14.23	
Cal 5	10000	40502	40810	40656	32.60	
Control 1	253 – 481	1426	1433	1429	1.15	322
Control 2	1008 – 1878	5541	5496	5519	4.43	1512
Sample 1		9243	9172	9207	7.38	2260
Sample 2		12288	12677	12482	10.01	3040
Sample 3		17896	18308	18102	14.51	4150

Example of a typical assay, 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

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 compounds was evaluated in this assay. The cross-reactivity was determined as the ratio of the apparent increase in IGFB-3 level (ng/ml) to the concentration of the potentially cross-reacting compound (ng/ml).

COMPOUND	Cross-reactivity (%)
IGFBP-1	0
IGFBP-2	0

10.2. Analytical sensitivity

The minimum detectable concentration of IGFBP-3 has been assayed at 50 ng/ml and corresponds to the concentration given by two standard deviation above the mean cpm of 20 replicates determinations of the zero calibrator.

10.3. Imprecision

Repeatability (Within assay variation) and reproducibility (Between assay variation)

	Mean value (ng/ml)	Within assay variation 20 replicates (% CV)	Between assay variation 10 separate assays (% CV)
Pool 1	756	10.2	12.4
Pool 2	1739	5.6	8.2
Pool 3	3780	4.6	6.3

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 IGFBP-3 (ng/ml)	Assayed IGFBP-3 (ng/ml)	IGFBP-3 recovered (ng/ml)	% of recovery
1040 + 3307	4347	3770	2730	82.5
1040 + 3720	4760	4115	3075	82.7
1040 + 4509	5549	5350	4310	95.6

10.5. Linearity : Dilution Test

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

Dilution factor	1/1	1/2	1/4	1/8
Assayed conc. (ng/ml)	5400	2594	1330	665
Expected conc. (ng/ml)	-	2700	1350	675

10.6. Interfering Substances

The ¹²⁵I tracer contains mouse normal serum to counteract the heterophilic antibodies and the rheumatoid factor.

11. Bibliography

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