IBL INTERNATIONAL

Instructions for Use

IGFBP-1 ELISA

Enzyme immunoassay for the direct quantitative determination of human Insulin like growth factor binding protein-1 in human serum.

REF **DB59131**

Σ **96**

2-8°C

EU: IVD CE

U.S.: *For research use only.*
Not for use in diagnostic procedures.

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INTENDED USE

For the direct quantitative determination of Insulin-Like Growth Factor Binding Protein-1 by enzyme immunoassay in human serum.
For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for IGFBP-1 is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of IGFBP-1 is conjugated to horse radish peroxidase (HRP). IGFBP-1 from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of IGFBP-1 in the sample.
A set of standards is used to plot a standard curve from which the amount of IGFBP-1 in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Insulin-like growth factor binding protein-1 (IGFBP-1) is one of six proteins that specifically bind insulin-like growth factors I and II (IGF-I and IGF-II) in body fluids and tissues.
IFGBP-1 contains 234 amino acids, with a predicted molecular mass of 25 kDa. The major sites of IGFBP-1 synthesis are the fetal /adult liver and decidualized endometrium.
Serum levels of IGFBP-1, which reflect its synthesis by the liver, exhibit considerable diurnal variation. Circulating IGFBP-1 levels are highest early in the morning and lowest in the evening. The levels are high in the fetus and newborn, but decline steadily until puberty. The mean level of IGFBP-1 in healthy adults is 4.4 µg/L (range 0.6-14.4 µg/L). After about 65 years of age, serum IGFBP-1 levels begin to increase. There is also an inverse correlation between body mass index (BMI) and fasting serum IGFBP-1 concentrations.
The most important regulator of circulating IGFBP-1 is insulin. Fasting insulin and IGFBP-1 concentrations are inversely correlated. During a 3-h glucose tolerance test, there is a decrease of about 50% in serum IFGBP-1 levels. Eating a meal also has a decreasing effect.
In insulin-dependent diabetes (IDDM), serum IGFBP-1 levels are elevated. In non-insulin dependent diabetes, in which insulin levels are high, serum IGFBP-1 is decreased. Low levels of IGFBP-1 have also been observed in the following cases: acromegaly, Cushing's syndrome and polysystic ovarian syndrome (PCO).

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a green colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of IGFBP-1 in human serum. The kit is not calibrated for the determination of IGFBP-1 in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and oxalic acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 20, 50, 80, 100 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Orbital shaker (600 rpm) (e.g. EAS 2/4, SLT) or Linear shaker (200rpm)
5. Microwell plate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 13).

REAGENTS PROVIDED

1. **Mouse Anti-IGFBP-1 Antibody Coated Microwell Plate-Break Apart Wells** - Ready To Use.
   Contents: One 96 well (12x8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.

2. **Mouse Anti-IGFBP-1 Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate X100** - Ready To Use.
   Contents: Anti-IGFBP-1 monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 250 µL/vial
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.

Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µL of HRP in 2 mL of buffer). If the whole plate is to be used dilute 120 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. **IGFBP-1 Calibrators** - Ready To Use.
   Contents: Six vials containing IGFBP-1 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of IGFBP-1.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 µg/L	2.0 mL
Calibrator B	1 µg/L	0.5 mL
Calibrator C	5 µg/L	0.5 mL
Calibrator D	30 µg/L	0.5 mL
Calibrator E	100 µg/L	0.5 mL
Calibrator F	250 µg/L	0.5 mL

   Storage: Refrigerate at 2-8°C
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** - Ready To Use.
   Contents: Two vials containing IGFBP-1 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of IGFBP-1. Refer to vial labels for expected value and acceptable range.
   Volume: 0.5 mL/vial
   Storage: Refrigerate at 2-8°C
   Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate - X10**
   Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **Assay Buffer** - Ready To Use.
   Contents: One vial containing a protein-based buffer with a non-mercury preservative.
   Volume: 26 mL/vial
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.

7. **TMB Substrate** - Ready To Use.
   Contents: One bottle containing Tetramethylbenzidine and hydrogen peroxide in a non-DMF oder DMSO containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2-8 °C
   Stability: 12 months or as indicated on label.

8. **Stopping Solution** - Ready To Use.
   Contents: One vial containing 1M sulfuric acid.
   Volume: 6 mL/vial
   Storage: Refrigerate at 2-8 °C
   Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: **None.**

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solution of the anti-IGFBP-1 conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µL of assay buffer into each well (We recommend using a multichannel pipette).
5. Incubate on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm) for 30 minutes at room.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 100 µL of the conjugate working solution into each well (We recommend using a multichannel pipette).
8. Incubate on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm) for 30 minutes at room..
9. Wash the wells again in the same manner as step 6.
10. Pipette 100 µL of TMB substrate into each well at timed intervals.
11. Incubate 10-15 min at RT (18-25°C) on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm) or until calibrator F attains dark blue colour for desired OD.
12. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 10.
13. Read the plate on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 415 nm filter is unavailable, a 405 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

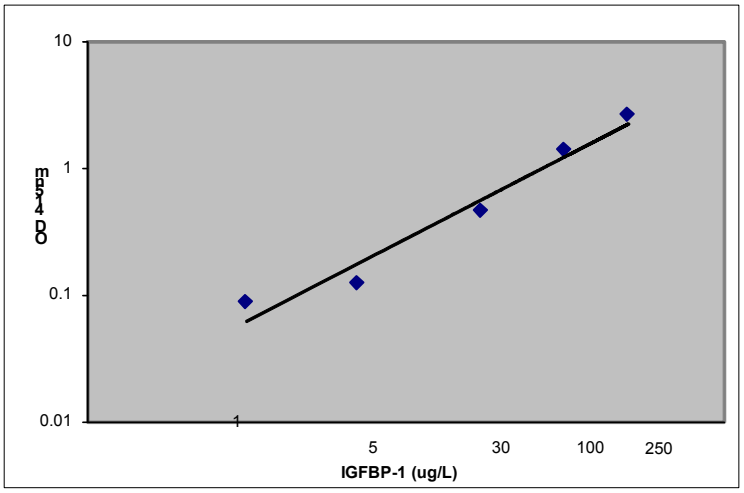
1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the “0” calibrator from the mean absorbance values of the calibrators, control and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 220 µg/L then dilute it with assay buffer at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (µg/L)
A	0.077	0.075	0.076	0
B	0.086	0.088	0.087	1
C	0.120	0.125	0.123	5
D	0.459	0.452	0.456	30
E	1.404	1.356	1.380	100
F	2.591	2.639	2.615	250
Unknown	0.120	0.117	0.119	4.5

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the IGFBP-1 ELISA kit is **0.5 µg/L.**

SPECIFICITY (CROSS REACTIVITY)

The specificity of the IGFBP-1 ELISA kit was determined by measuring the apparent IGFBP-1 value of calibrator A spiked with the following compounds:

Substance	Concentration Range	Apparent IGFBP-1 Value (µg/L)
IGFBP-2	Up to 5000 µg/L	Not Detected
IGFBP-3	Up to10,000 µg/L	Not Detected
IGFBP-4	Up to 5000 µg/L	Not Detected
IGFBP-5	Up to 5000 µg/L	Not Detected

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µg/L) are tabulated below:

Sample	Mean	SD	CV%
1	5.5	0.14	2.5
2	22	0.75	3.4
3	117	2.8	2.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µg/L) are tabulated below:

Sample	Mean	SD	CV%
1	4.8	0.31	6.4
2	21	1.6	7.4
3	113	5.6	4.9

RECOVERY

Spiked samples were prepared by adding defined amounts of IGFBP-1 to three patient serum samples (1:1). The results (in µg/L) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	5.0	-	-
+6.5	5.8	5.75	100.9
+35	20	20	100.0
+174	90	89.5	100.6
2 Unspiked	20	-	-
+6.5	14	13.3	105.3
+35	29	24.5	118.4
+174	100	97	103.1
3 Unspiked	110	-	-
+6.5	62	58.3	106.3
+35	80	72.5	110.3
+174	155	133	116.5

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µg/L) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	13.5	-	-
1:2	6.9	6.8	101.5
1:5	3.4	3.4	100.0
1:10	1.6	1.4	114.3
2	38	-	-
1:2	20.9	19	110.0
1:5	8.2	7.6	107.9
1:10	4.2	3.8	110.5
3	120	-	-
1:2	58.2	60	97.0
1:5	22.1	24	92.1
1:10	11.5	12	95.8

HIGH DOSE HOOK EFFECT

The IGFBP-1 ELISA kit did not experience a high dose hook effect when it was tested up to an IGFBP-1 concentration of 200,000 µg/L.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Mean (µg/L)	Abs. Range (µg/L)
Adults	55	4.4	0.6-14.4

REFERENCES

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3. Rutanen E-M et al. Relationship Between Carbohydrate Metabolism and Serum Insulin-Like Growth Factor System in Postmenopausal Women: Comparison of Endometrical Cancer Patients with Healthy Controls. J. Clin. Endocrinol. Metab. 77: 1999-204, 1993.
4. Rutanen E-M et al. Measurement of Insulin-Like Growth Factor Binding Protein-1 in Cervical/Vaginal Secretions: Comparison with the ROM-Check Membrane Immunoassay in the Diagnosis of Ruptured Fetal Membranes. Clin. Chim. Acta. 214:73-81, 1993.
5. Lockwood et al. Fetal Membrane Rupture is Associated with the Presence of Insulin-Like Growth Factor Binding Protein-1 in Vaginal Secretions. Am. J. Obstet. Gynecol. 171: 146-150, 1994.

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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