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

Instructions for use

IGFBP-1 ELISA

REF **ME E-0400**

IGFBP-1 ELISA

INTENDED USE

For the direct quantitative determination of Insulin-Like Growth Factor Binding Protein-1 by an enzyme immunoassay in human serum.
For *in vitro* 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 microplate 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 Standard curve must be established for every run.
7. The controls 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 controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells 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 blue 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.

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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 Standard 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 controls has been tested and found to be nonreactive 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. No test method however, 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 sulfuric 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. Plate shaker
5. Microplate 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. AA E-0030 **WASH-CONC** **10x** **Wash Buffer Concentrate** – Requires Preparation **X10**

Content:	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.

2. AA E-0055 **SUBSTRATE** **TMB Substrate** – Ready To Use

Content:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	16 ml/bottle
Storage:	Refrigerate at 2 – 8 °C
Stability:	12 months or as indicated on label.

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3. AA E-0080 **STOP-SOLN** **Stopping Solution** – Ready To Use

Content: One vial containing 1 M sulfuric acid.

Volume: 6 ml/bottle

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months or as indicated on label.

Hazards identification:

H315 Causes skin irritation.

H319 Causes serious eye irritation.

4. Standards and **Controls** – Ready To Use

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

Cat. no.	Symbol	Standard	Concentration	Volume/Vial
ME E-0401	**STANDARD A**	**Standard A**	0 µg/l	2.0 ml
ME E-0402	**STANDARD B**	**Standard B**	1 µg/l	0.5 ml
ME E-0403	**STANDARD C**	**Standard C**	5 µg/l	0.5 ml
ME E-0404	**STANDARD D**	**Standard D**	30 µg/l	0.5 ml
ME E-0405	**STANDARD E**	**Standard E**	100 µg/l	0.5 ml
ME E-0406	**STANDARD F**	**Standard F**	250 µg/l	0.5 ml
ME E-0451	**CONTROL 1**	**Control 1**	Refer to vial labels for expected value and acceptable range!	0.5 ml
ME E-0452	**CONTROL 2**	**Control 2**		0.5 ml

Content: IGFBP-1 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of IGFBP-1.

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.

5. ME E-0413 **ASSAY-BUFF** **Assay Buffer** – Ready To Use

Content: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 26 ml/bottle

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months or as indicated on label.

6. ME E-0431 **96** **Mouse Anti-IGFBP-1 Antibody-Coated Break-Apart Well Microplate** – Ready To Use

Content: One 96 well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months or as indicated on label.

7. ME E-0440 **CONJUGATE-CONC** **100x** **Mouse Anti-IGFBP-1 Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate** – Requires Preparation **X100**

Content: 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 assay 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.

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ASSAY PROCEDURE

(Specimen Pretreatment: None)
All reagents must reach room temperature before use. Standards, 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 solutions** of the **anti-IGFBP-1 conjugate** and **wash buffer.**
2.	Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3.	Pipette **25 µl** of each **Standard, Control** and **specimen samples** into correspondingly labelled wells in duplicate.
4.	Pipette **100 µl** of the **assay buffer** into each well. *(We recommend using a multichannel pipette.)*
5.	Incubate on a plate shaker (approximately 200 rpm) for **30 minutes** at **room temperature**.
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 a plate shaker (approximately 200 rpm) for **30 minutes** at **room temperature**.
9.	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.)*
10.	Pipette **100 µl** of **TMB substrate** into each well at timed intervals.
11.	**Incubate** on a plate shaker for **10 – 15 minutes** at room temperature. *(or until Standard 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 microplate 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 450 nm filter is unavailable, a 405 or 415 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 Standard duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the Standard A from the mean absorbance values of the Standards, controls and serum samples.
4. Draw a Standard curve on log-log paper with the mean optical densities on the Y-axis and the Standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the Standard curve.
6. If a sample reads more than 220 µg/l, then dilute it with Standard A at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

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

Standard	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

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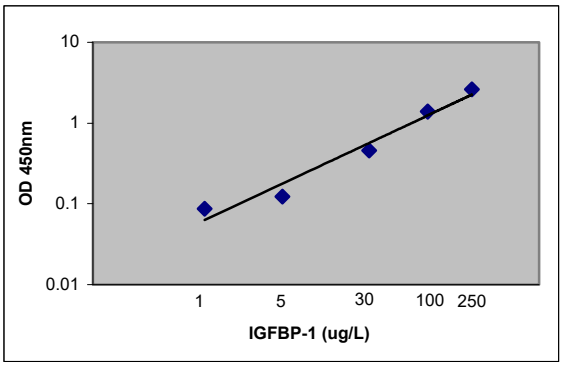
TYPICAL STANDARD CURVE

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 Standard 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 Standard 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 to 10,000 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 Standard 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

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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 Standard 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

1. Rutanen EM. Insulin-Like Growth Factor Binding Protein-1 (Review) *Seminars in Reprod Endocrinol*. 1992; 10:154–63.
2. Rutanen EM, et al. Aging is Associated with Decreased Suppression of Insulin-Like Growth Factor Binding Protein-1 by Insulin. *J Clin Endocrinol Metab*. 1993; 77(5):1152–5.
3. Rutanen EM, 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*. 1993; 77(1):199–204.
4. Rutanen EM, 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*. 1993; 214(1):73–81.
5. Lockwood CJ, 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*.1994; 171(1):146–50.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE marking of conformity
	Caution	REF	Catalogue number		Distributor
	Date of manufacture				