

Instruction Manual No. M-900

**Human Insulin-Like Growth Factor
Binding Protein-1 (IGFBP-1)**

**For Quantitative Determination of IGFBP-1
Human In Serum**

For In Vitro Research Use Only

ELISA KIT Cat. No. 0900

For Quantitative Determination of IGFBP-1 In Human Serum

Kit Contents: (reagents for 96 tests)

| Components | 96 tests |
|--|-----------|
| Anti-IGFBP coated strip plate (96 wells), Cat. # 9 0 1 | 1 plate |
| IGFBP1 Std. A , 0.0 ug/L; 2 ml, Cat # 902 | 1 Vial |
| IGFBP1 Std. B , 1 ug/L; 0.5 ml, Cat # 903 | 1 Vial |
| IGFBP1 Std. C , 5.0 ug/L; 0.5 ml, Cat # 904 | 1 Vial |
| IGFBP1 Std. D , 30 ug/L; 0.5 ml, Cat # 905 | 1 Vial |
| IGFBP1 Std. E , 100 ug/L; 0.5 ml, Cat # 906E | 1 Vial |
| IGFBP1 Std. F , 250 ug/L; 0.5 ml, Cat # 906F | 1 Vial |
| Assay Buffer, 26 ml; Cat # 907 | 1 bottle |
| Anti-hIGFBP1-HRP Conjugate Conc., 250 ul, cat # 908 | 1 Vial |
| IGFBP1 Control serum, see vial for exact value | 1 Vial |
| Wash buffer concentrate (10X solution), 50 ml | Cat.#W-10 |
| TMB substrate , 13 ml, Cat. # S - 2 0 | 1 bottle |
| Stop solution (oxalic acid), Cat. # T - 3 0 | 1 bottle |
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Introduction

IGFBP-1 is one of six proteins that specifically bind IGF-1 and IGF-2. IGFBP-1 contains 234 aa (~25 kda). It is synthesized in the liver and decidualized endometrium. Serum levels of IGFBP-1 are high 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 levels of IGFBP-1 in healthy adults are 4.4 ug/L (range 0.6-14.4 ug/L). After about 65-yrs, IGFBP-1 levels begin to increase. There is also inverse relationship between body mass index (BMI) and fasting serum levels. IGFBP-1 is regulated by insulin. Fasting insulin and IGFBP-1 concentrations are inversely related. During a 3-h glucose tolerance test, there is a decrease of about 50% in serum IGFBP-1. Its concentration decrease after a meal. In insulin dependent diabetes mellitus (IDDM), IGFBP-1 is elevated. In non-insulin IDDM, IGFBP-1 is decreased due to elevated insulin concentration. Low levels of IGFBP-1 are also observed in acromegaly, Cushing's syndrome and polycystic ovarian syndrome (PCO).

PRINCIPLE OF THE TEST

IGFBP-1 ELISA test is based on sequential binding of human IGFBP-1 from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (green color) is directly proportional to the amount of IGFBP-1 present in the sample. The reaction is terminated by adding stopping solution (color stays dark green). Absorbance is then measured on a microtiter well ELISA reader at 414 nm. The unknown sample values are then read-off the standard curve.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicates determinations of the zero standard, the minimum IGFBP-1 concentration detectable using this assay is 0.4 ug/L. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision: Three serum samples (mean IGFBP-1 concentrations: 5.5, 22, and 117 µg/L) were run in sixteen replicates. The samples showed good intra-assay precision with %CV of 2.5, 3.4, and 2.4, respectively.

Inter-assay precision: Three serum samples were run in duplicate in fifteen independent assays. The samples showed good inter-assay precision (5-7.4 % CV). The actual values were: mean 4.8 µg/L, SD 0.31 µg/L, %CV 6.4; mean 21 µg/L, SD 1.6 µg/L, %CV 7.4; mean 113 µg/L, SD 5.6 µg/L, %CV 4.9, respectively.

3. RECOVERY

A known amount of IGFBP-1 (6.5, 35, and 174 µg/L) was added to three patient sera (with original IGFBP-1 concentrations of 5, 20, and 110 µg/L) and the total IGFBP-1 concentrations measured. The assay showed excellent mean recoveries of about 105% (100-110%).

4. LINEARITY

Five different patient samples (with original IGFBP-1 concentrations of 5.4, 13.5, 38, 94, and 120 µg/L) were diluted (1:2, 1:5, and 1:10) with the zero standard and their final IGFBP-1 values determined. The samples showed excellent mean recoveries of about 102% (range 92-111%). Patient sample with an original concentration of 5.4 µg/L IGFBP-1 could not be detected when diluted 1:10.

5. SPECIFICITY

The specificity of IGFBP-1 ELISA kit was determined by measuring interference from high concentrations of human placental Lactogen, HCG, human prolactin, and human AFP. These hormones had a minimal interference in the BP-1 assay (0.01% or less). No measurable interference was detected from IGBP-2-5 (5000-10000 ug/L).

References: Rutanen EM et al (1992) Sem. Reprod. Endo. 10, 154; Rutanen EM (1993) J Clin. Endocrinol. Metabol. 77, 1152; Rutanen EM (1993) J Clin. Endocrinol. Metabol. 77, 1999; Lockwood et al (1994) Am. J. Obstet. Gynecol. 171, 146

Citation of ADI's IGFBP-1 ELISA kit-

Krikun G2004 Endocrinology, Jan 2004 in Press.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Standards are stable for two months at 2-8°C. Reconstituted control serum is stable for one week at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 µl of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Dilute **wash buffer** concentrate 1:10 with water, dilute **HRP conjugate conc. 1:100 with assay buffer** before use.
2. Label or mark the microtiter well strips to be used on the plate.
3. Pipet **25 µl of standards**, control, and serum samples into appropriate wells in *duplicate*.
4. Pipet **100 µl of assay** buffer into each well. Cover the plate and incubate at room temperature for **30 minutes** on a plate shaker (about 250 rpm; failure to shake will decrease kinetics).
5. Aspirate and **wash the wells 3-5 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
6. Add **100 µl of diluted enzyme conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temperature on a plate shaker (about 150 rpm; failure to shake will decrease kinetics).
7. Aspirate and wash the wells 3 X with diluted wash buffer, as above. Add **100 µl of HRP-substrate (TMB)** at timed intervals into each well. Blue color develops in positive wells.
8. Cover the plate and incubate at room temperature for **10-15 min** on a plate shaker (about 150 rpm; failure to shake will decrease color). Absorbance (A450) of the highest standards must not exceed the linear range of reader (typically 2.0-3.0).
9. Stop the reaction by adding **50 µl of stop solution** to all wells at the same timed intervals as in step 8. Mix gently. Blue color turns yellow.
10. Measure **absorbance at 450 nm** using an ELISA reader. Color is stable for at least one hr after stopping.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision.

Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples containing more than 150 µg/L IGFBP-1 must be diluted with the zero standard (standard A) and the results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples.

Draw the standard curve on log-log graph paper by plotting net absorbance values of standards on the y-axis and the IGFBP-1 concentrations on the x-axis. Read off the IGFBP-1 concentrations of the control and patient samples. If a software is used, 4-parameter curve is recommended.

All samples with a reading of >220 µg/L must be diluted 1:10 with the zero standard and re-assayed. Results obtained must be multiplied by the dilution factor.

Normal Values

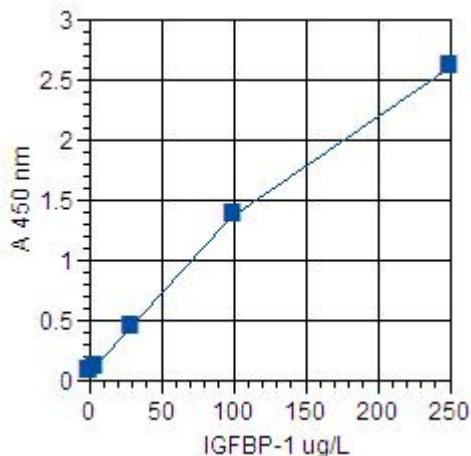
As for all clinical assays, each laboratory must establish their own normal range. The following values were established using ADI's ELISA kit.

| | | |
|---------------------|-----------------|-----------------------|
| Adults (55 samples) | Mean (4.4 µg/L) | Range (0.6-14.4 µg/L) |
|---------------------|-----------------|-----------------------|

WORKSHEET OF TYPICAL ASSAY

| Wells | Stds/samples | Mean A _{414 nm} | Calculated Concn (µg/L) |
|--------|-------------------|--------------------------|-------------------------|
| A1, A2 | Std. A (0 ug/L) | 0.076 | |
| B1, B2 | Std. B (1.0 ug/L) | 0.087 | |
| C1, C2 | Std. C (5.0 ug/L) | 0.123 | |
| D1, D2 | Std. D (30 ug/L) | 0.456 | |
| E1, E2 | Std. E (100 ug/L) | 1.380 | |
| F1, F2 | Std. F (250 ug/L) | 2.615 | |
| G1, G2 | Sample 1 | 0.119 | 4.5 |

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips.

Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate reader.

PRECAUTIONS

The IGFBP-1 ELISA kit is intended for *in vitro research* use only. The reagents contain thimerosal and gentamicin as preservatives; necessary care should be taken when disposing solutions. The Control and Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Avoid contact with the reagents (Substrate, stop solution). ABTS substrate is a suspected carcinogen. All safety precautions must be used in disposing the chemical waste.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

This kit is not optimized for saliva, plasma, or other fluids. Samples containing azide or thimerosal may lead to false results.

Presence of heterophiles antibodies or patients that have been injected with antibodies or other animal products may have the potential to interfere in the assay. Similarly, some individuals may have antibodies to the protein (IGFBP-1) that may also interfere in the assay.

REAGENTS PREPARATION FOR THE ASSAY

IGFBP-1 Standards Calibrated against Behring Institute IGFBP-1 (PP12) preparation Lot 307/323. The standard values are about 0, 1, 5, 30, 100 and 250 ug/L. Exact standard values are given on each vial.

Dilute wash buffer 1:10 with water. Occasionally, crystal may form in the concentrate but they will dissolve upon warming and mixing.