ELISA kits available from ADI (see details at the web site)

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin 
#1200 Human Albumin (Urinary)
#1750 Human IgG (total) 
#1760 Human IgM
#1800 Human IgE 
#1810 Human Ferritin
#1210 Human Transferrin (Tf) 
#0020 Beta-2 microglobulin
#1660 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) 
#1830 Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) 
#1510 free PSA (PSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin 
#0040 Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) 
#0410 HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) 
#1110 Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) 
#1700 Human T3 (total)

#1850 Human Cortisol 
#1860 Human Progesterone
#1865 Human Pregnolone 
#1875 Human Aldosterone
#1880 Human Testosterone 
#1885 Human free Testosterone
#1910 Human Androstenedione 
#1920 Human Estradiol
#1925 Human Estrone 
#1940 Dihydrotestosterone (DHT)
#1950 Human DHEA-sulphate (DHEA-S)
#3400 Human serum Neopterin

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA
#3200 Anti-Nuclear Antibodies (ANA)

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

ELISA KIT  Cat. No. 900

For Quantitative Determination of IGFBP-1
Human In Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email:  service@4adi.com
Web Site:  www.4adi.com
ELISA KIT Cat. No. 0900
For Quantitative Determination of IGFBP-1 In Human Serum

Kit Contents: (reagents for 96 tests)

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

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 IGFBP-2-5 (5000-10000 µg/L).


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 month 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 ul 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 ul of standards, control, and serum samples into appropriate wells in duplicate.
4. Pipet 100 ul 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 ul 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 ul 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 ul 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 ul 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 ug/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 ug/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 ug/L)  Range (0.6-14.4 ug/L)
**WORKSHEET OF TYPICAL ASSAY**

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean A414 nm</th>
<th>Calculated Concn (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Std. A (0 µg/L)</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>Std. B (1.0 µg/L)</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>Std. C (5.0 µg/L)</td>
<td>0.123</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>Std. D (30 µg/L)</td>
<td>0.456</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Std. E (100 µg/L)</td>
<td>1.380</td>
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</tr>
<tr>
<td>F1, F2</td>
<td>Std. F (250 µg/L)</td>
<td>2.615</td>
<td></td>
</tr>
<tr>
<td>G1, G2</td>
<td>Sample 1</td>
<td>0.119</td>
<td>4.5</td>
</tr>
</tbody>
</table>

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.

**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 Alpha Diagnostic International 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 µg/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.