**INTENDED USE**

The IGFBP-2 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of IGFBP-2 in human serum. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

**SUMMARY AND EXPLANATION**

IGFBP-2 is an unglycosylated polypeptide of 31.3 kDa and is one of the six circulating proteins that bind insulin-like growth factor (IGF)-I and -II with high affinity. Cleavage of signal peptide from the precursor protein generates the 31 kDa mature IGFBP-2, which consists of N- and C-terminal cysteine rich regions (1,2). Both N- and C-terminal regions of IGFBP-2 bind IGF-I and IGF-II preventing their interaction with IGF-1 receptor (2). IGFBP-2 mainly exists in non-glycosylated and non-phosphorylated form (3,4).

IGFBP-2 is a widely expressed protein with functions in bone and skeletal muscle development, and regulation of body growth and composition (5). Pure congenic IGFBP2 -/- mice show gender and bone compartment-specific phenotypes (6). IGFBP-2 overexpression mice have reduced body weight suggesting a role in postnatal growth by potentially regulating IGF-I bioavailability (7).

IGFBP-2 levels in circulation are influenced under different metabolic and malignant states (5). IGFBP-2 levels are high in cord serum from term infants, in contrast plasma IGFBP-2 levels in adults are lower (8). IGFBP-2 levels in adults are subjected to minimal daily fluctuations suggesting that postprandial changes in glucose and insulin levels do not influence IGFBP-2 levels (8). Compared to normal adults, IGFBP-2 levels are elevated in hypopituitary adults suggesting regulation by growth hormone (8). Extreme physical exercise results in elevated serum IGFBP-2 levels in men and women (5). Circulating IGFBP-2 levels are high in anorexia nervosa patients and suppressed in obesity and Type 2 diabetes (5). Overexpression of IGFBP-2 protects against obesity and diabetes by inhibiting adipogenesis and modulating insulin sensitivity (9,10). In contrast to Type 2 diabetes, IGFBP-2 levels are increased in Type 1 diabetes suggesting regulation by insulin sensitivity (11). Low postpartum IGFBP-2 levels are associated with the development of Type 2 diabetes in women with history of gestational diabetes mellitus (12).

IGFBP-2 levels are also altered in intra-uterine growth retardation (IUGR) and small for gestational age (SGA) cases. In IUGR cord serum levels of IGFBP-2 are elevated and levels are low in pre-pubertal and pubertal SGA subjects (5). IGFBP-2 role in cancer progression is indicated by elevated levels in several malignancies like prostate, ovarian, breast and gastric cancer (5). In colon cancer IGFBP-2 levels are elevated in plasma and is associated with increased risk of mortality (13). Recently, IGFBP-2 was identified as an early stage biomarker in invasive ductal adenocarcinoma of pancreas (14) and elevated serum IGFBP-2 levels were reported in idiopathic pulmonary fibrosis and Lupus nephritis patients (15,16). Circulating levels of IGFBP-2 may be an important biomarker of different metabolic and malignant states.

**PRINCIPLE OF THE TEST**

The IGFBP-2 is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to IGFBP-2 antibody coated micro titer wells and incubated. After the first incubation, and washing, the wells are incubated with biotinylated IGFBP-2 antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 550 nm as reference filter. The absorbance measured is directly proportional to the concentration of IGFBP-2 in the samples and calibrators.

**MATERIALS SUPPLIED**

**CAL-140A** IGFBP-2 Calibrators A/Sample Diluent
One bottle, 10 mL, labeled IGFBP-2 Cal A/Sample Diluent, containing 0 ng/mL IGFBP-2 in a buffer and Pro-Clean 400. Store unopened at 2-8°C until the expiration date.

**CAL-140B -- Cal-140F** IGFBP-2 Calibrators B – F
Five vials, labeled B-F, containing concentrations of approximately 0.45-16 ng/mL IGFBP-2 in a buffer. Refer to calibration card for exact concentrations. Store unopened at -20°C until the expiration date. Avoid repeated freeze thaws. The IGFBP-2 concentration in the IGFBP-2 calibrators is traceable to the manufacturer’s working calibrators. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

**CTR-140-I & CTR-140-II** IGFBP-2 Controls I & II
Two vials, labeled Levels I and II containing low and high IGFBP-2 concentrations in buffer. Refer to calibration card for exact concentrations. Store unopened at -20°C until the expiration date. Avoid repeated freeze thaws.

**PLT-140** IGFBP-2 Coated Microtiter strips
One strip holder, containing 12 strips and 96 microtiter wells with IGFBP-2 antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.
For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

**SAMPLE COLLECTION AND PREPARATION**

a) Serum is the recommended sample type. Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer’s instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

b) Samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

c) Avoid assaying lipemic, hemolyzed or icteric samples.

d) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

e) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

**PROCEDURAL NOTES**

1. A thorough understanding of this package insert is necessary for successful use of the IGFBP-2 ELISA assay. It is the user’s responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.

2. A calibration curve must be included with each assay.

3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.

4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.

5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

**PREPARATION OF REAGENTS**

1. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.

2. **IGFBP-2 Antibody-Biotin Conjugate Solution:** The IGFBP-2 Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1 part conjugate to 50 parts of IGFBP-2 Assay buffer, according to the number of wells used. If an entire plate is to be used pipet exactly 220 μL of the Concentrate in to 11 mL of the Assay buffer.

3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from contamination, sodium azide, and a non-mercury mercury preservative.

**WARNINGS AND PRECAUTIONS**

For Research Use Only. Not for use in diagnostic procedures.

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

**WARNING: Potential Biohazardous Material**

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.

**WARNING: Potential Chemical Hazard**

Some reagents in this kit contain Pro-Clean 400 and Sodium azide as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.
ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicate.

**Note:** All Male, Female and First Trimester samples should be diluted 1:10 (10µL sample+ 90µL IGFBP-2 Calibrator A/Sample Diluent). Samples from Second Trimester onwards should be run neat. Do not dilute the calibrators and controls.

1. Allow the calibrators to reach the room temperature (23 ± 2°C) & mix well by gentle vortex.
2. Label the microtiter strips to be used.
3. Pipette 25 µL of the Calibrator, Controls and Unknowns to the appropriate wells.
4. Add 100 µL of the IGFBP-2 Assay Buffer to each well using a repeater pipette.
5. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 60 minutes at room temperature (23 ± 2°C).
6. With 30-40 minutes remaining of incubation time, prepare the IGFBP-2 Antibody-Biotin Conjugate Solution by diluting the IGFBP-2 Biotin Conjugate Concentrate in IGFBP-2 Assay buffer as described under the Preparation of the Reagents section of this insert.
7. Aspirate and wash each strip 5 times with Wash Solution (350 µL/per well) using an automatic microplate washer.
8. Add 100 µL of the IGFBP-2 Biotin Conjugate solution to each well using a repeater pipette.
9. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 60 minutes at room temperature (23 ± 2°C).
10. Aspirate and wash each strip 5 times with the Wash Solution (350 µL/per well) using an automatic microplate washer.
11. Add 100 µL of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
12. Incubate the plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 30 minutes at room temperature (23 ± 2°C).
13. Aspirate and wash each strip 5 times with the Wash Solution (350 µL/per well) using an automatic microplate washer.
14. Add 100 µL of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
15. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker, for 8-10min at room temperature (23 ± 2°C).
   **Note:** Visually monitor the color development to optimize the incubation time.
16. Add 100 µL of the stopping solution to each well using a precision pipette. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm.
   **Note:** While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a “Blank”.

RESULTS

**Note:** The results in this package insert were calculated by plotting the data on a log vs. log scale using a cubic regression curve-fit. Other data reduction methods may give slightly different results.

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the IGFBP-2 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
3. Determine the IGFBP-2 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding IGFBP-2 concentrations.
4. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (CAL A / Sample Diluent) and re-assayed.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. Multiply the value by a dilution factor.

LIMITATIONS

The reagents supplied in this kit are optimized to measure IGFBP-2 levels in human serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- The IGFBP-2 ELISA Controls or other commercial controls should fall within established confidence limits.
- The confidence limits for IGFBP-2 controls are printed on the calibration card.
- A full calibration curve, low and high level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

<table>
<thead>
<tr>
<th>Well Number</th>
<th>Well Contents</th>
<th>Mean Absorbance</th>
<th>Conc (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Calibrators A</td>
<td>0.026(Blank)</td>
<td>0</td>
</tr>
<tr>
<td>B1, B2</td>
<td>B</td>
<td>0.052</td>
<td>0.45</td>
</tr>
<tr>
<td>C1, C2</td>
<td>C</td>
<td>0.107</td>
<td>1.2</td>
</tr>
<tr>
<td>D1, D2</td>
<td>D</td>
<td>0.340</td>
<td>3.2</td>
</tr>
<tr>
<td>E1, E2</td>
<td>E</td>
<td>1.193</td>
<td>7.8</td>
</tr>
<tr>
<td>F1, F2</td>
<td>F</td>
<td>3.499</td>
<td>16</td>
</tr>
</tbody>
</table>

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in ng/mL.

Analytical Sensitivity:
The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviations of 21 runs with two replicates each (n=42) of calibrator A (0 ng/mL) and Calibrator B (0.45ng/mL) is 0.08 ng/mL.

Imprecision:
Reproducibility of the IGFBP-2 assay was determined in a study using two controls. The study included a total of 21 assays, two replicates of each per assay (n=24). Representative data were calculated based on NCCLS EPS-A guidelines and are presented in the following table.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean conc.</th>
<th>Within run</th>
<th>Between run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/mL)</td>
<td>SD %CV</td>
<td>SD %CV</td>
<td>SD %CV</td>
</tr>
<tr>
<td>Control I</td>
<td>1.38</td>
<td>0.037</td>
<td>2.71%</td>
<td>0.029</td>
</tr>
<tr>
<td>Control II</td>
<td>5.14</td>
<td>0.233</td>
<td>4.53%</td>
<td>0.188</td>
</tr>
</tbody>
</table>
Sample Stability:
Twenty-two serum samples were compared in the IGFBP-2 ELISA assay from day 0 (fresh draw)- day 59 for stability.
The following box plots represent serum concentrations at different time intervals and conditions of the samples.

![Graph showing serum IGFBP-2 levels over time](image)

**Linearity:**
Based on dilutions of the three serum samples containing various IGFBP-2 levels diluted with Calibrator A/sample diluent the % recovery on individual samples is represented in the following:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>Expected Conc. (ng/mL)</th>
<th>Observed Conc. (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1, 1:10</td>
<td>Neat</td>
<td>15.94</td>
<td>7.69</td>
<td>97%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:20</td>
<td>7.97</td>
<td>3.66</td>
<td>92%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:40</td>
<td>3.97</td>
<td>1.83</td>
<td>92%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:80</td>
<td>1.99</td>
<td>1.83</td>
<td>92%</td>
</tr>
<tr>
<td>S-2, 1:10</td>
<td>Neat</td>
<td>20.00</td>
<td>10.87</td>
<td>109%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:20</td>
<td>10.00</td>
<td>5.25</td>
<td>105%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:40</td>
<td>5.00</td>
<td>2.43</td>
<td>97%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:80</td>
<td>2.50</td>
<td>2.43</td>
<td>97%</td>
</tr>
<tr>
<td>S-3, 1:10</td>
<td>Neat</td>
<td>19.9</td>
<td>10.80</td>
<td>108%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:20</td>
<td>9.96</td>
<td>5.21</td>
<td>105%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:40</td>
<td>4.98</td>
<td>2.42</td>
<td>98%</td>
</tr>
<tr>
<td>Neat</td>
<td>1:80</td>
<td>2.49</td>
<td>2.42</td>
<td>98%</td>
</tr>
</tbody>
</table>

**Recovery:**
Known amounts of IGFBP-2 were added to four serum samples containing different levels of endogenous IGFBP-2. The concentration of IGFBP-2 was determined before and after the addition of exogenous IGFBP-2 and the percent recovery was calculated.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endogenous Conc.(ng/mL)</th>
<th>Expected Conc. (ng/mL)</th>
<th>Observed Conc. (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.30</td>
<td>3.67</td>
<td>3.59</td>
<td>98%</td>
</tr>
<tr>
<td>2</td>
<td>0.80</td>
<td>3.43</td>
<td>3.51</td>
<td>102%</td>
</tr>
</tbody>
</table>

**Cross reactivity and specificity:**
The IGFBP-2 antibody pair detects human, canine, bovine and equine, caprine and rat species. Other related analytes at the concentration in the table below did not show any cross-reactivity.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Cross-reactant</th>
<th>Concentration (ng/mL)</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat IGF-I</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>IGF-I/IGFBP-3 complex</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>IGF-II</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>human IGF-I</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>IGFBP-3</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>IGFBP-4</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>IGFBP-5</td>
<td>1000</td>
<td>ND</td>
</tr>
</tbody>
</table>

**ND** = Non Detectable

**Interference:**
When potential interferents (hemoglobin, triglycerides and bilirubin) were added at least at two times their physiological concentration to control sample, IGFBP-2 concentration were within ± 10% of the control as represented in the following table.

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Analyte Conc. (mg/mL)</th>
<th>Unspiked Sample Value (ng/mL)</th>
<th>Spiked Sample Value (ng/mL)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>1.35</td>
<td>145.22</td>
<td>144.47</td>
<td>-0.52</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>5.00</td>
<td>205.35</td>
<td>203.22</td>
<td>-1.04</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.60</td>
<td>198.84</td>
<td>198.84</td>
<td>-0.00</td>
</tr>
</tbody>
</table>

**REFERENCES**

14. Yoneyama T, Ohtsuki S, Honda K et al. Identification of IGFBP2 and IGFBP3 As Compensatory Biomarkers for CA19-9 in Early-Stage Pancreatic Cancer Using A Combination of Antibody-Based and LC-MS/MS-Based Proteomics. PLoS One. 2016;11(8)
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