

## Human IGFBP-3 ELISA Kit (hIGFBP-3-ELISA)

*Cat. No. EK0386*

*96 Tests in 8 x 12 divisible strips*

### Background

Insulin-like growth factor-binding protein-3 (IGFBP-3) is a major determinant of circulating levels of the IGFs and is clinically useful for the evaluation of GH deficiency and for predicting the response to GH treatment. The circulating level of IGFBP-3 is inversely related to the risk of several common cancers, and antiproliferative agents such as antiestrogens and retinoids act in part by up-regulating IGFBP-3 expression. IGFBP-3, well-characterized as the carrier of IGF, has been reported to have intrinsic bioactivity that is independent of IGF binding; IGFBP-3 has an IGF-independent, antiproliferative effect in undifferentiated and early differentiated, but not in terminally differentiated, chondrocytes. IGFBP-3 possesses both growth-inhibitory and potentiating effects on cells that are independent of IGF action and are mediated through specific IGFBP-3 binding proteins/receptors located at the cell membrane, cytosol, or nuclear compartments and in the extra cellular matrix. IGFBP-3 is located on chromosome 7. The standard product used in this kit is recombinant human IGFBP-3, consisting of 265 amino acids with the molecular mass of 29kDa. As a result of glycosylation, the molecular mass is 41kDa.

ScienCell's human IGFBP-3 ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Human IGFBP-3 specific polyclonal antibodies are pre-coated onto 8 x 12 divisible strips. The human specific detection polyclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of human IGFBP-3 captured in strips.

<b>Size</b>	96 Tests 8×12 divisible strips
<b>Assay type</b>	Sandwich ELISA
<b>Range</b>	156 pg/ml - 10,000 pg/ml
<b>Sensitivity</b>	< 10 pg/ml
<b>Specificity</b>	No detectable cross-reactivity with any other cytokine.
<b>Storage</b>	Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles.
<b>Shipping</b>	Shipped on gel ice.

<b>Expiration</b>	Four months at 4°C and eight months at -20°C.
<b>Application</b>	For quantitative detection of human IGFBP-3 in serum, plasma, body fluids, tissue lysates or cell culture supernatants.
<b>Kit components</b>	<ol style="list-style-type: none"> <li>1. Lyophilized recombinant human IGFBP-3 standard: 10 ng/tube×2.</li> <li>2. 8 x 12 divisible strips pre-coated with anti- human IGFBP-3 antibody.</li> <li>3. Sample diluent buffer: 30 ml</li> <li>4. Biotinylated anti-human IGFBP-3 antibody: 130µl, dilution 1:100.</li> <li>5. Antibody diluent buffer: 12ml.</li> <li>6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.</li> <li>7. ABC diluent buffer: 12 ml</li> <li>8. TMB color developing agent: 10 ml.</li> <li>9. TMB stop solution: 10 ml.</li> </ol>
<b>Materials</b>	1. Microplate reader.
<b>Required But Not Provided</b>	<ol style="list-style-type: none"> <li>2. Automated plate washer.</li> <li>3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.</li> <li>4. Clean tubes and Eppendorf tubes.</li> <li>5. Washing buffer (neutral PBS or TBS).</li> </ol> <p>Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.</p> <p>Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.</p>
<b>Usage</b>	This product is for research use only. It is not approved for use in humans, animals, or <i>in vitro</i> diagnostic procedures.

## Reference

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2. Spagnoli, A.; Torello, M.; Nagalla, S. R.; Horton, W. A.; Pattee, P.; Hwa, V.; Chiarelli, F.; Roberts, C. T., Jr.; Rosenfeld, R. G. Identification of STAT-1 as a molecular target of IGFBP-3 in the process of chondrogenesis. *J. Biol. Chem.* 277: 18860-18867, 2002.
3. Weinzimer, S. A.; Gibson, T. B.; Collett-Solberg, P. F.; Khare, A.; Liu, B.; Cohen, P. Transferrin is an insulin-like growth factor-binding protein-3 binding protein. *J. Clin. Endocr. Metab.* 86: 1806-1813, 2001.
4. Ehrenborg, E.; Larsson, C.; Stern, I.; Janson, M.; Powell, D. R.; Luthman, H. Contiguous localization of the genes encoding human insulin-like growth factor-binding proteins 1 (IGBP1) and 3 (IGBP3) on chromosome 7. *Genomics* 12: 497-502, 1992.

## Protocol for Human IGFBP-3 ELISA (96 well format)

### Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.
5. Do not let strips dry, as this will inactivate active components in wells.
6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

## **Preparation**

### **Sample Preparation and Storage**

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

- **Cell culture supernatants, tissue lysates or body fluids:** Remove particulates by centrifugation, assay immediately or aliquot and store at -20°C.
- **Serum:** Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 minutes. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma:** Collect plasma using heparin, EDTA as an anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as the anticoagulant.

### **Sample Dilution Guideline**

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

- **High target protein concentration (100-1000 ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (10-100 ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (156-10,000 pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration ( $\leq 156$  pg/ml).** No dilution necessary, or the working dilution is 1:2.

### **Reagent Preparation and Storage**

A. Reconstitution of the human IGFBP-3 standard: IGFBP-3 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of IGFBP-3 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10,000 pg/ml of human IGFBP-3 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.
- 5000pg/ml→156pg/ml of human IGFBP-3 standard solutions: Label 6 Eppendorf tubes with 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10,000pg/ml IGFBP-3 standard solution into 1<sup>st</sup> tube and mix.

Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3<sup>rd</sup> tube and mix, and so on.

**Note:** The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human IGFBP-3 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume).
- Biotinylated anti-human IGFBP-3 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume).
- Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

### **Assay Procedure**

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard IGFBP-3 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IGFBP-3 amount in samples.

1. Aliquot 0.1ml per well of the 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml human IGFBP-3 standard solutions into the pre-coated strips. Add 0.1ml of the sample diluent buffer into the control well (**blank well**). Add 0.1ml of each properly diluted sample of human serum, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “**Sample Dilution Guideline**” above for details. We recommend that each human IGFBP-3 standard solution and each sample is measured in duplicate.
2. Seal the strips with the cover and incubate at 37°C for 90 minutes.
3. Remove the cover, discard strips’ contents, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human IGFBP-3 antibody working solution into each well and incubate the strips at 37°C for 60 minutes.
5. Wash strips 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method:** Discard the solution in the strips without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of **THREE** washes. Note: For automated washing, aspirate all wells and wash **THREE** times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 minutes.
7. Wash strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material.(See Step 5 for strips washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate strips at 37°C in dark for 15-20 minutes (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades

of blue can be seen in the wells with the four most concentrated human IGFBP-3 standard solutions; the other wells show no obvious color).

9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human IGFBP-3 concentration of the samples can be interpolated from the standard curve.

**Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

### Summary

1. Add samples and standards and incubate the strips at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 minutes. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 minutes. Wash strips 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the strips at 37°C in dark for 15-20 minutes.
5. Add TMB stop solution and read.

### **Typical Data Obtained from Human IGFBP-3**

(TMB reaction incubate at 37°C for 15 minutes)

Concentration (pg/ml)	0.0	156	312	625	1250	2500	5000	10,000
Absorbance (450 nm)	0.043	0.103	0.148	0.239	0.422	0.809	1.460	2.420

### **Typical Human IGFBP-3 ELISA Kit Standard Curve**

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

