HUMAN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP-3) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN IGFBP-3 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN IGFBP-3 ELISA
Catalog No.	SK00053-02
Lot No.	20111147
Formulation	96 T
Standard range	62.5 - 8000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL of Diluted Sample
Dilution Factor	250 to 500-Fold (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human IGFBP-3
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8°C

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Human IGFBP-3 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human IGFBP-3 in cell culture supernatants, serum, and EDTA plasma. It contains recombinant human IGFBP-3 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human IGFBP-3. Results obtained with naturally occurring IGFBP-3 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human IGFBP-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IGFBP-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IGFBP-3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for IGFBP-3 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of IGFBP-3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_Centrifuge vials prior to assay.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
IGFBP-3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IGFBP-3.	**053-02-01**	**1 plate**
IGFBP-3 Standard – 8000 pg/vial of recombinant human IGFBP-3 in a buffered protein base with preservative; lyophilized.	**053-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against human IGFBP-3 with preservative; lyophilized.	**053-02-03**	**1 vial**
Positive Control – one vial of recombinant human IGFBP-3, lyophilized.	**053-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservative.	**DB17**	**2 bottles**
Wash Buffer - 50mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C

or -70 °C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect from light) and other components may be stored at 2 – 8 °C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 – 8 °C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma and serum samples may need a 250 or 500-fold dilution. A suggested 10-fold dilution is 10 µL sample + 90 µL Dilution Buffer. To make a 250-fold dilution, add 10 µL of 10-fold diluted sample into 240 µL Dilution Buffer. To make a 500-fold dilution, add 5 µL of 10-fold diluted sample into 245 µL Dilution Buffer. **Optimal dilutions should be determined and optimized by each laboratory for each application.**
Use polypropylene test tubes.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

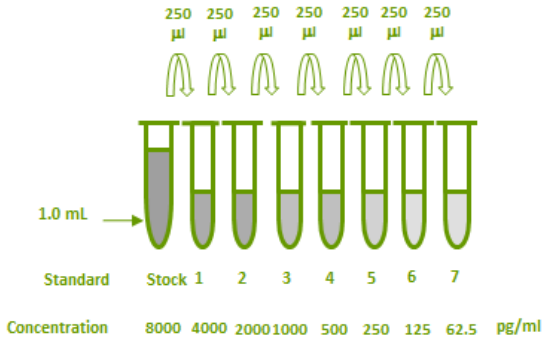
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

IGFBP-3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **IGFBP-3** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1.0 mL	8000 pg/mL
# 1	250µL of stock	250µL	4000 pg/mL
# 2	250µL of 1	250µL	2000 pg/mL
# 3	250µL of 2	250µL	1000 pg/mL
# 4	250µL of 3	250µL	500 pg/mL
# 5	250µL of 4	250µL	250 pg/mL
# 6	250µL of 5	250µL	125 pg/mL
# 7	250µL of 6	250µL	62.5 pg/mL



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

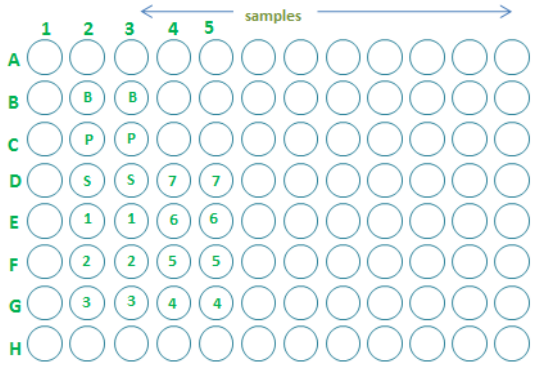
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).

Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used within a few days if stored at -20 °C or -70 °C.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of **Dilution Buffer** to blank wells (B2, B3).
4. Add 100 µL of **Standard** in reverse order of serial dilution (D4, D5 to G4, G5 and G2, G3 to D2, D3), **sample**, or **positive control** (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 4-8 minutes on micro-plate shaker at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting

the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IGFBP-3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Readings of sample concentration exceeding that of the standard 8000 pg/mL may result in inaccurate, low human IGFBP-3 levels. Such samples require further external pre-dilution according to expected human IGFBP-3 values with Dilution Buffer in order to precisely quantify the actual human IGFBP-3 level.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

IGFBP-3 STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.061)
31.25 (optional)	0.017
62.5	0.032
125	0.061
250	0.124
500	0.244
1000	0.482
2000	0.899
4000	1.674
8000	2.593

- Lot No.: 20111147
- Positive Control: 100 – 300 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human IGFBP-3.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of IGFBP-3 was 15 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human IGFBP-3. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEINS	CROSS-REACTIVITY
Human IGFBP-3	100%
Human IGF-I R	0
Human IGFBP-1	0
Human IGFBP-2	0
Human IGFBP-4	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard, samples, positive control to each well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin-HRP Conjugate working solution to each well. Incubate 60 min on plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 4-8 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min.