HUMAN EPIGEN (EPGN)/EPITHELIAL MITOGEN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN EPGN CONCENTRATIONS IN SERUM AND EDTA PLASMA

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN EPGN ELISA
Catalog No.	SK00340-01
Lot No.	
Formulation	96 T
Standard range	31 - 2000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Type	Serum and EDTA Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human EPGN
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
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 Epigen (EPGN) immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human EPGN in serum and EDTA plasma. It contains recombinant human EPGN and antibodies raised against this protein. It has been shown to accurately quantify recombinant human EPGN. Results obtained with naturally occurring EPGN 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 EPGN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for EPGN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any EPGN present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for EPGN is added to the wells. Following a wash to remove any unbound antibody, Goat Anti Rabbit IgG-HRP 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 EPGN bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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.

_ 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 the appropriate 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
EPGN Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against EPGN.	**340-01-01**	**1 plate**
EPGN Standard – 8000 pg/vial of recombinant human EPGN in a buffered protein base with preservatives; lyophilized.	**340-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of polyclonal antibody against EPGN with preservatives; lyophilized.	**340-01-03**	**1 vial**
Positive Control - one vial of recombinant human EPGN; lyophilized	**340-01-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Goat Anti Rabbit IgG HRP conjugate with preservatives	**ARIGHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 mL 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 8 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. ARIG-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 - 8°C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

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.

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.

SAMPLE COLLECTION AND STORAGE

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, heparin, or citrate 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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

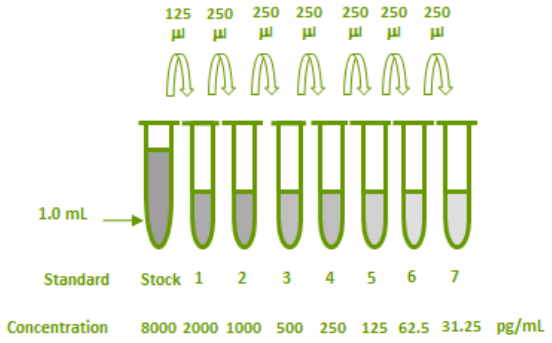
REAGENT PREPARATION

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 Wash Buffer.

EPGN Standard - Refer to vial label for reconstitution volume. Reconstitute the **EPGN 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 375 µL of Dilution Buffer into tube #1 and transfer 125 µL of stock solution to prepare the maximum standard solution of 2000 pg/mL. Pipette 250 µL of Dilution Buffer into tubes #2 to #7. Use the **2000 pg/mL** solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	8000 pg/ml
# 1	125 µl of stock	375 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl of 3	250 µl	250 pg/ml
# 5	250 µl of 4	250 µl	125 pg/ml
# 6	250 µl of 5	250 µl	62.5 pg/ml
# 7	250 µl of 6	250 µl	31.25 pg/ml



Positive Control – Reconstitute the **Positive Control** with 1.0 mL Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

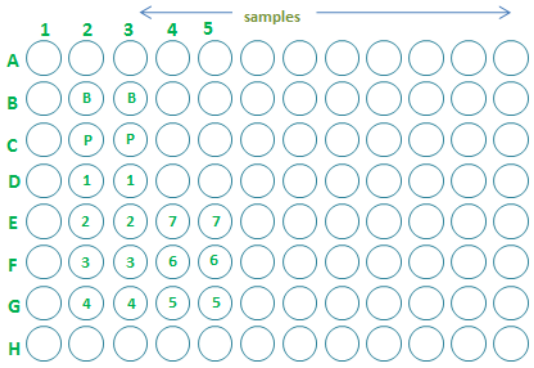
Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Anti Rabbit IgG-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days **(protect from light).**

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 (from E4, E5 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 Anti Rabbit IgG-HRP Conjugate working solution to each well. Incubate for 1 hour 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 8-12 minutes on a 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 EPGN 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.

TYPICAL DATA

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

EPGN (pg/mL)	Average OD450 (Corrected)*
Blank	0 (0.118)
15.625 (optional)	0.021
31.25	0.043
62.5	0.080
125	0.127
250	0.407
500	0.505
1000	1.043
2000	1.917

- Lot No.:
- Positive Control: 250 - 600 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human EPGN.

SENSITIVITY

The minimum detectable dose (MDD) of EPGN was 10 pg/mL.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human EPGN	100
Human HGF	0
Human HGF-alpha	0
Human VEGF-R1	0
Human TNF-alpha	0
Human IL-6	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 the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Anti Rabbit IgG HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate solution to each well. Incubate 8-12 min on plate shaker. **Protect from light**.
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min