HUMAN PRO BMP8 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN PRO BMP8 CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

THIS IS ONE TIME USE ONLY.

PRODUCT INFORMATION:

ELISA NAME	**HUMAN PRO BMP8 ELISA**
Catalog No.	SK00017-01
Lot No.	
Formulation	96 T
Standard range	3.125 - 100 ng/mL
Sensitivity	0.7 ng/mL
Sample Volume	100 µL
Dilution Factor	**2-4 (Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum and Plasma
Specificity	Human Pro BMP8
Calibration	Human Pro-BMP8 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8°C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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

DESCRIPTION

This Human Pro BMP8 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Pro-BMP8from serum and plasma samples in a sandwich ELISA format. Other sample types need to be validated with this assay.

This immunoassay contains recombinant human Pro BMP8 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Pro BMP8 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for Pro BMP8. The capture antibody can bind to the Pro BMP8 in the standard and samples. After washing the plate of any unbound substances, a polyclonal antibody against Pro BMP8-IIB is added to the wells. After another washing of the plate, a secondary antibody-HRP conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of Pro BMP8 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Pro BMP8 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Pro BMP8.	**017-01-01**	**1 plate**
Pro BMP8 **Standard –** 100 ng/vial of recombinant human Pro BMP8 in a buffered protein base with preservative; lyophilized.	**017-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of a purified polyclonal antibody against Pro BMP8 with preservative; lyophilized.	**017-01-03**	**1 vial**
Positive Control - one vial of recombinant human ACTR-IIB; lyophilized.	**017-01-04**	**1 vial**
Anti-Rabbit IgG-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Goat Anti-Rabbit IgG conjugated to HRP.	**ARIGHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
Antibody Diluent Solution- 30 mL of buffered protein based solution with preservative.	**DB40**	**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 solution.	**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°C or -70°C. Do not use kit past expiration date.
This is one time use elisa kit. Please DO NOT reuse any reconstituted components.
Microplate Wells: Return unused wells to the plastic pouch (P01) with desiccant pack. Microplate may be stored at 2 – 8°C for up to 6 months after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel pipette, pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	125 ng/ml
# 1	250µl of stock	250µl	62.5 ng/ml
# 2	250µl of 1	250µl	31.25 ng/ml
# 3	250µl of 2	250µl	15.625 ng/ml
# 4	250µl of 3	250µl	7.813 ng/ml
# 5	250µl of 4	250µl	3.906 ng/ml
# 6	250µl of 5	250µl	1.953 ng/ml

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 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

Serum and plasma samples require 2-4 fold dilution.
Optimal dilutions should be determined by each laboratory for each application with a pretest.
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 1x Wash Buffer.

Pro BMP8 Standard - Reconstitute the ACTR-IIB standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 100 ng/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 #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **100 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20°C ~ -70°C.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Antibody Diluent Solution to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Anti-Rabbit IgG-HRP Conjugate - Pipette 11.88 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **(protect from light).**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all samples, reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of standard dilutions in reverse order of serial dilution from #6 to #S, samples, or positive control per well. Cover with the plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 the plate sealer. Incubate for 2 hours on microplate 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 60 minutes on microplate 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 5-10 minutes on microplate 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 microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

The samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor (pretreated as suggested will have a dilution factor of 4).

Sample O.D. readings with a concentration exceeding that of standard 125 ng/mL may result in inaccurate, low human ACTR-IIB levels. Such samples require further external predilution according to expected human ACTR-IIB values with Dilution Buffer and then pretreated in order to precisely quantify the actual human ACTR-IIB level.

TYPICAL STANDARD CURVE

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.112)
1.56 (optional)	0.016
3.125	0.031
6.25	0.069
12.5	0.159
25	0.301
50	0.562
100	1.099

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Pro BMP8	100%
Human BMP9	0
Human BMP4	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard dilutions, samples, or 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 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 5-10 min on the plate shaker at RT. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read 450 nm within 15 min.