

HUMAN BONE MORPHOGENETIC PROTEIN 9 (BMP9) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN BMP9 CONCENTRATIONS IN
CELL CULTURE SUPERNATES, EDTA PLASMA
AND SERUM



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

PURCHASE INFORMATION:

ELISA NAME	HUMAN BMP9 ELISA
Catalog No.	SK00139-01
Lot No.	
Formulation	96T
Standard range	7.8-500 pg/mL
Sensitivity	3.9 pg/mL
Sample Volume	100 µl
Sample Type	Cell Culture Supernates, EDTA Plasma and Serum
Dilution Factor	(Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human BMP9
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8°C

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INTRODUCTION

Human BMP9 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human BMP9 in cell culture supernates, EDTA plasma and serum. It contains recombinant human BMP9 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human BMP9. Results obtained with naturally occurring BMP9 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 BMP9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for BMP9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BMP9 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for BMP9 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 BMP9 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.
- _ 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
BMP9 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against BMP9.	139-01-01	1 plate
BMP9 Standard – 2500pg/vial of recombinant human BMP9 in a buffered protein base with preservatives; lyophilized.	139-01-02	1 vial
Detection Antibody Concentrate – 110 µL/vial, 100-fold concentrate of biotinylated polyclonal antibody against BMP9 with preservatives; lyophilized.	139-01-03	1 vial
Positive Control - one vial of recombinant human BMP9, lyophilized	139-01-04	1 vial
Streptavidin-HRP Conjugate - 75 ul/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer - 60mL of buffered protein based solution with preservatives	DB01	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 piece

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°C or -70°C. Do not use kit past expiration date.
Opened/Reconstituted Reagents: Reconstituted Standard and Detection Antibody SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated

solution and other components may be stored at 2-8°C for up to 6 months.

Microplate Wells: Return unused wells to the plastic pouch containing the desiccant pack, reseal 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, with the correction wavelength set at 540 nm or 570 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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ 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 $\leq -20^{\circ}$ 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 $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

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

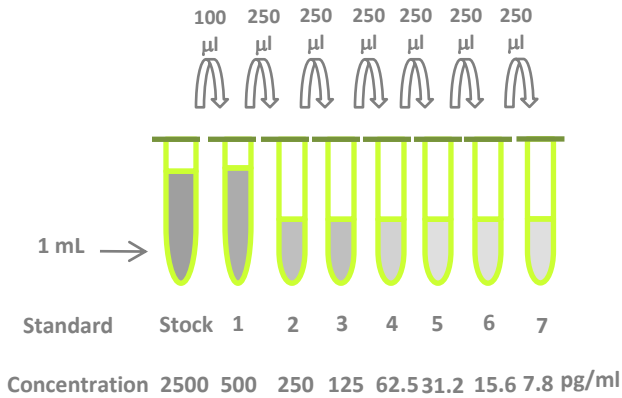
REAGENT PREPARATION

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.

BMP9 Standard - Refer to vial label for reconstitution volume. Reconstitute the **BMP9 Standard** with 1.0 ml of Dilution Buffer. This reconstitution produces a stock solution of 2500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 400 μ L of Dilution Buffer into tube #1, then transfer 100 μ L of stock solution to prepare high standard of 500 pg/mL. Pipette 250 μ L of Dilution Buffer into tubes #2 to #7. Use the high standard solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 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 μ l	2500 pg/ml
# 1	100 μ l of stock	400 μ l	500 pg/ml
# 2	250 μ l of 1	250 μ l	250 pg/ml
# 3	250 μ l of 2	250 μ l	125 pg/ml
# 4	250 μ l of 3	250 μ l	62.5 pg/ml
# 5	250 μ l of 4	250 μ l	31.25 pg/ml
# 6	250 μ l of 5	250 μ l	15.6 pg/ml
# 7	250 μ l of 6	250 μ l	7.8 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 110 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.89 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 110 µL of 100-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.*

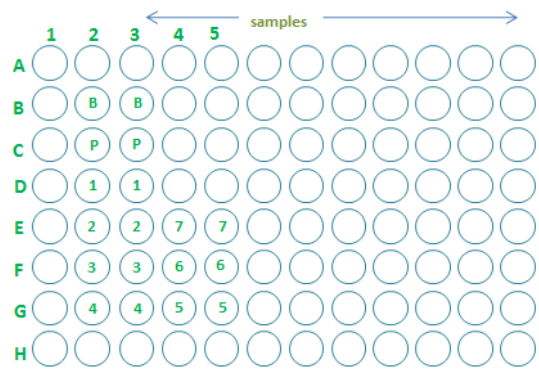
Positive Control - Reconstitute the **Positive Control** with 1.5 mL of Dilution Buffer. *Positive Control should be prepared and used immediately.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards 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 containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard (D2, D3 to G2, G3 and E4, E5 to G4, G5), sample, or positive control (C2, C3) per well. Cover with 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 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 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 12-18 minutes 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- logistic 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 BMP9 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.

BMP9 (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.126)
7.8	0.049
15.6	0.106
31.2	0.203
62.5	0.401
125	0.798
250	1.587
500	2.713

*Lot No.:

** Positive Control: 100-200 pg/ml

CALIBRATION

This immunoassay is calibrated against a highly purified CHO-expressed recombinant human BMP9.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of BMP9 was 3.9 pg/mL.

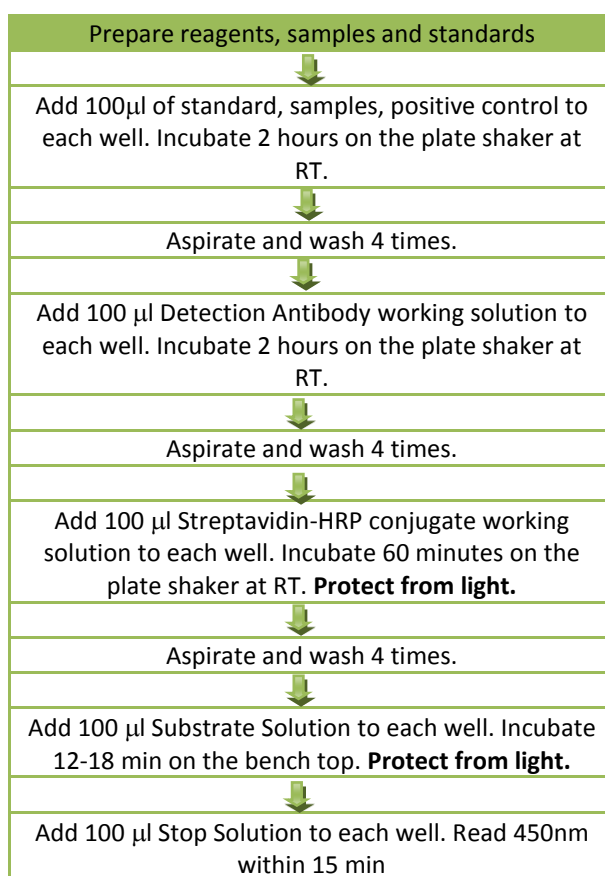
SPECIFICITY

This assay recognizes both natural and recombinant human BMP9. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh BMP9 control were assayed for interference. No significant cross-reactivity or interference was observed.

Human Recombinant Proteins:

BMP3, BMP4, BMP5, BMP6, BMP7, BMP10, BMP8, TGF-beta1.

SUMMARY OF ASSAY PROCEDURE



REFERENCES:

- David L, et al. Bone morphogenetic protein-9 is a circulating vascular quiescence factor. Circ Res. 2008 Apr 25;102(8):914-22. Epub 2008 Feb 28.