Human BMP-2 Immunoassay

Catalog Number: SEKH-0105

For the quantitative determination of Human BMP-2 concentrations in cellculture supernate, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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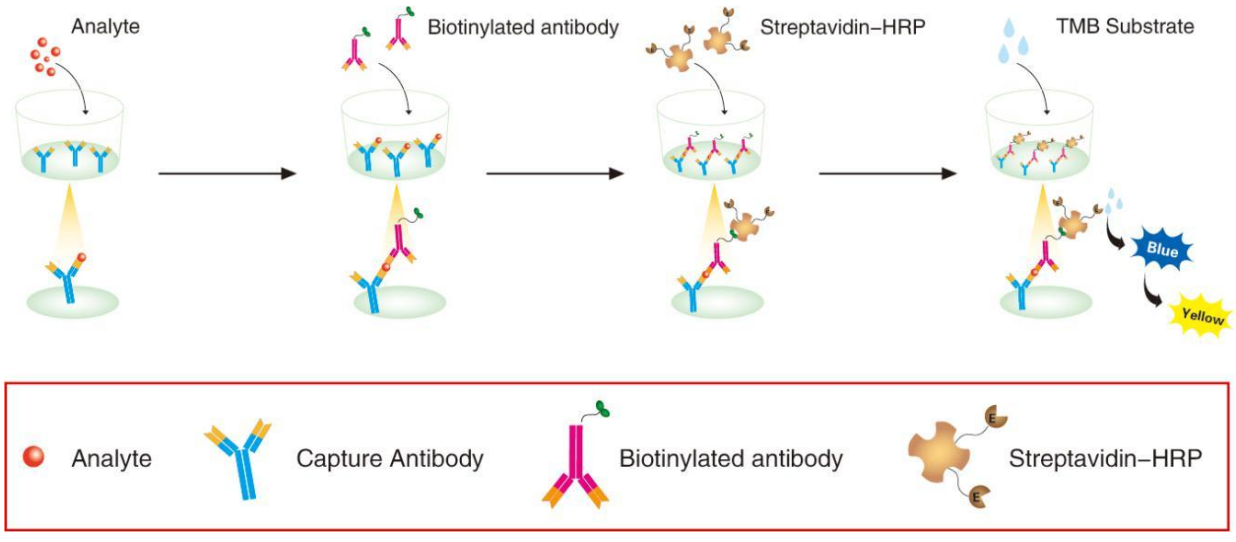
BACKGROUND

Bone morphogenetic protein-2 (BMP-2, previously known as BMP-2A) is a member of thetransforming growth factor beta (TGF-β) superfamily, based on amino acid (aa) sequencehomology. BMPs were originally identified as protein regulators of cartilage and boneformation. They have also been implicated in embryogenesis and morphogenesis of varioustissues and organs.They can regulate growth, differentiation, chemotaxis and apoptosis of avariety of cell types, including mesenchymal, epithelial, hematopoietic and neuronal cells.BMP-2 has pleiotropic functions including organogenesis, bone formation and regeneration,and regulation of pattern formation in the developing limb bud. Recombinant humanBMP-2 has been shown to possess potent ectopic bone forming activity in a variety ofexperimental systems.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for BMP-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BMP-2 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for BMP-2 is added to detect the captured BMP-2 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugatedStreptavidinis added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTSAND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,3000 pg/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
ConcentratedBiotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
ConcentratedStreptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard/Sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent- 16ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

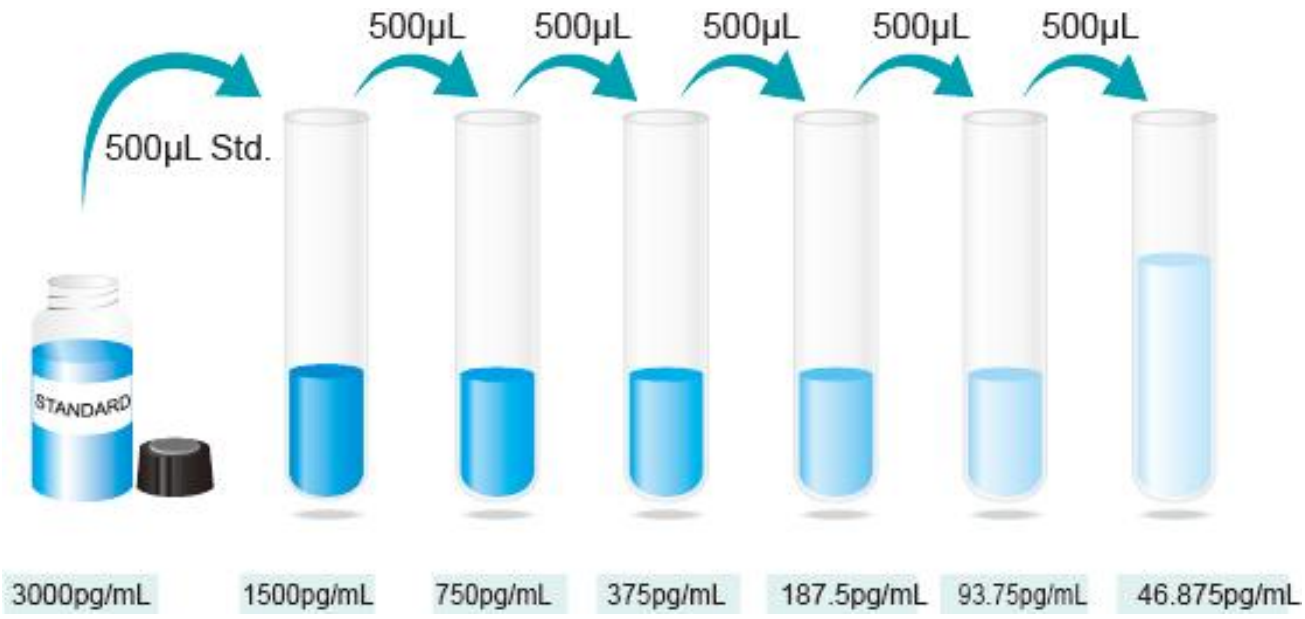
Cell Culture Supernate- Centrifuge cell culture media at 1000×gto remove debris. Assay immediately oraliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at roomtemperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assayimmediately 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 for15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and storesamples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentratewith 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mixgently until the crystals have completely dissolved.

3. **Standard\Sample** -Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 3000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Sample Diluent into 1500pg/ml tube and the remaining tubes. Use the stock solution of 3000pg/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 3000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Human BMP-2 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human BMP-2 antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100µl standard or samples to each well,shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human BMP-2 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 10-30 minutes (depending on signal),at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

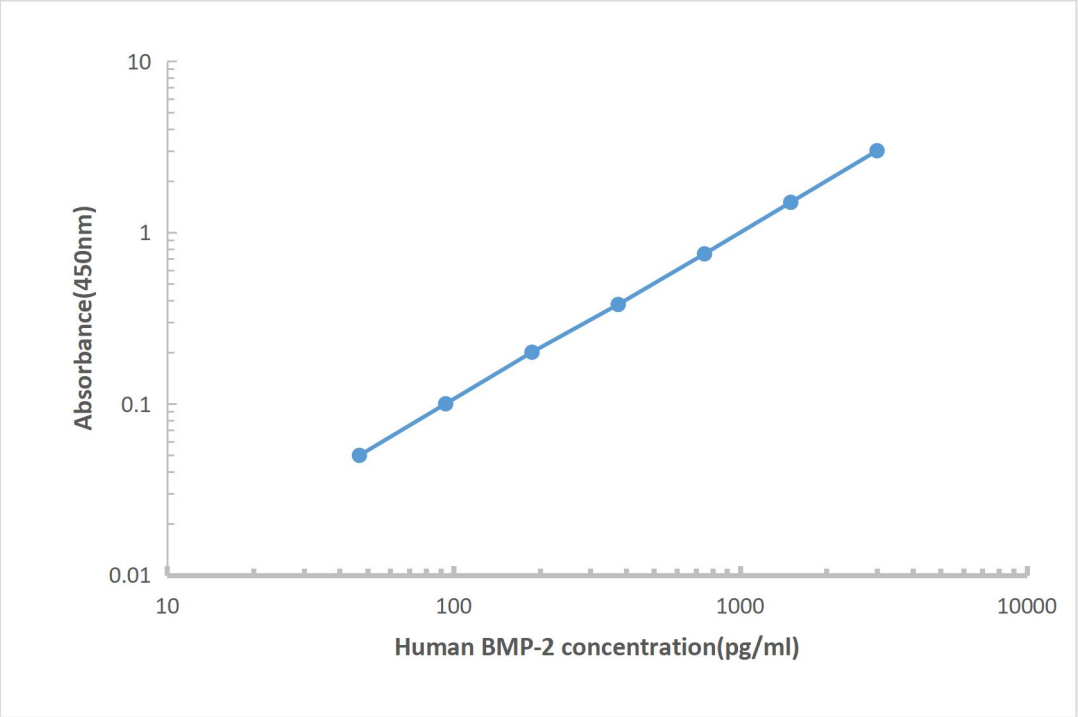
1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample.All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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.
4. The data may be linearized by plotting the log of the BMP-2concentrations 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.

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

Typical data using the BMP-2 ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.057	0.053	-
46.88	0.183	0.175	0.179	0.127
93.8	0.267	0.255	0.261	0.208
188	0.396	0.379	0.388	0.335
375	0.630	0.603	0.616	0.564
750	1.023	0.979	1.001	0.948
1500	1.665	1.594	1.629	1.577
3000	2.713	2.597	2.655	2.602



Representative standard curve for BMP-2ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 23pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human BMP-2. The factors listed below were prepared at 100ng/ml in Standard /Sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
Activin A	BMPR-IB	
ctivin RIA	Follistatin	
Activin RIIA		
Activin RIIB		
BMP-2/BMP-7 Heterodimer		
BMP-5		
BMP-6		
BMP-7		
BMPR-IB		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of BMP-2 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of BMP-2 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	93	83-101
Cell culture supernatants	96	85-103

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of BMP-2in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	94	105
	Range (%)	86-104	95-113
1:4	Average% of Expected	95	107
	Range (%)	84-102	96-113

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