

BMP-2 (human) ELISA Kit

(Catalog # K4744-100, 100 assays; Store at -20°C)

rev 1/14

I. Introduction:

BioVision's Human BMP-2 (Bone morphogenetic proteins-2) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human BMP-2. This assay employs an antibody specific for human BMP-2 coated on a 96-well plate. Standards and samples are pipetted into the wells and BMP-2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human BMP-2 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of BMP-2 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. The minimum detectable dose of BMP-2 is typically less than 45 pg/ml. The intra-Assay reproducibility is CV<10% & inter-Assay is CV<12%. In this ELISA format, the pair antibody shows less than 1% cross-reactivity with rhBMP-3, rhBMP-4, rhBMP-5, rhBMP-6 or rhBMP-7.

II. Application:

Quantitative protein detection, establishing normal range, validation of antibody array results.

III. Specificity:

The antibody pair provided in this kit recognizes human BMP-2.

IV. Sample Type:

- Serum & plasma
- Cell culture supernatants
- Urine

V. Kit Contents:

Components	K4744-100	Part No.
BMP-2 Microplate (Item A): 96 wells, coated with anti-human BMP-2	12 stripsx8 wells	K4744-100-1
Wash Buffer Concentrate (20x) (Item B)	25 ml	K4744-100-2
Standards (Item C) (recombinant human BMP-2)	2 vials	K4744-100-3
Assay Diluent A (Item D) for Standard/Sample (serum/plasma) diluent (0.09% sodium azide as preservative)	30 ml	K4744-100-4
Assay Diluent B (Item E) for Standard/Sample (cell culture medium/urine) diluent. (5x concentrated)	15 ml	K4744-100-5
Detection Antibody BMP-2 (Item F), biotinylated anti-human BMP-2 (each vial enough for half plate)	2 vial	K4744-100-6
HRP-Streptavidin Concentrate (Item G), 200x concentrated	200 µl	K4744-100-7
TMB One-Step Substrate Reagent (Item H) 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution	12 ml	K4744-100-8
Stop Solution (Item I), 0.2 M sulfuric acid	8 ml	K4744-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

VII. Storage and Handling:

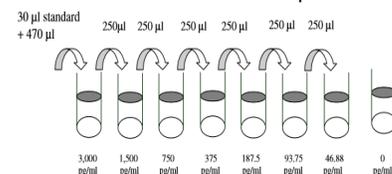
May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. **Note:** the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

VIII. Reagent Preparation:

- Bring all reagents and samples to room temperature (18 - 25°C) before use.
- Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 2 fold*.

*Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

- Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
- Preparation of standard: Briefly spin the vial of Item C then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 500 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 30 µl BMP-2 standard (50 ng/ml) from the vial of Item C, into a tube with 470 µl Assay Diluent A or 1x Assay Diluent B to prepare a 3000 pg/ml stock standard solution. Pipette 250 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 ng/ml).
- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
- Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Assay Protocol.



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- Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 200-fold with 1x Assay Diluent B.

IX. Assay Protocol:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette 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.
4. Add 100 µl of 1x prepared biotinylated antibody (see Reagent Preparation) to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
5. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation) to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
7. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.
8. Calculation: Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

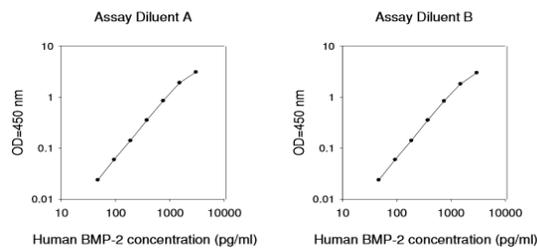


Figure 1: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

Sample Type	Average % Recovery	Range (%)
Serum	100.3	74-127
Plasma	98.46	70-130
Cell culture media	146.1	131-159

Table 1: Recovery: Recovery was determined by spiking various levels of human BMP-2 into human serum, plasma and cell culture media.

Sample Type	Serum	Plasma	Cell Culture Media
1:2 Average % of Expected Range (%)	76.94 69-85	101.1 94-105	88.31 78-98
1:4 Average % of Expected Range (%)	74.23 67-80	102.4 96-113	87.23 79-95

Table 2: Linearity

X. RELATED PRODUCTS:

BMP-2, Human Cell^{EXP} human recombinant
 BMP-4, Human Cell^{EXP} human recombinant
 Human IL-17F ELISA kit
 Human Insulin ELISA kit
 Recombinant proteins and enzymes
 Metabolism Assay kits and Reagents
 Protein A/G/L & Affinity Conjugates

BMP-2 Antibody
 Human IL-17 ELISA kit
 Human IL-15 ELISA kit
 Polyclonal and monoclonal antibodies
 Apoptosis Assay Kits and Reagents
 Cellular Fractionation Kits
 Recombinant Growth Factors and Cytokines

FOR RESEARCH USE ONLY! Not to be used on humans.