



Biotinylated Anti-human BMP-4 Antibody

ORDERING INFORMATION

Catalog Number: BAM7572

Clone: 66108

Lot Number: FMX02

Size: 250 µg

Formulation: 0.2 µm filtered solution in PBS with BSA

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human BMP-4

Immunogen: NS0-derived rhBMP-4

Ig class: mouse IgG₁

Recommended Applications:

Western blot
ELISA detection

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant human Bone Morphogenic Protein 4 (rhBMP-4). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography and then biotinylated. BMP-4 is one of at least 20 structurally and functionally related BMPs which are members of the TGF-β superfamily. Biologically active BMP-4 is a disulfide-linked homodimer of the carboxy-terminal domain. The morphogenetic effects of BMP-4 on various tissues are mediated by type I and type II BMP receptors.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) containing 50 µg/mL of bovine serum albumin per 1 µg of antibody.

Reconstitution

Reconstitute with sterile PBS. If 0.5 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody was selected for its ability to detect human BMP-4 in western blots and for use as a detection antibody in human BMP-4 sandwich ELISAs. In western blots, this antibody does not cross-react with rhBMP-2, -3, -5, -6, or -7.

Applications

Western Blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human BMP-4. Using a colorimetric detection system, the detection limit for rhBMP-4 is approximately 25 ng/lane and 5 ng/lane under non-reducing and reducing conditions, respectively. Chemiluminescent detection with WesternGlo Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) will increase sensitivity by 5 to 50 fold.

ELISA Detection - This antibody can be used as a detection antibody in a human BMP-4 sandwich ELISA in combination with human BMP-4 monoclonal capture antibody (Catalog # MAB7571). A general protocol is provided on the next page. Using plates coated with 100 µL/well of the capture antibody, in combination with 100 µL/well of the detection antibody at 1 µg/mL, an ELISA for sample volumes of 100 µL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 1 ng/mL.

Optimal dilutions should be determined by each laboratory for each application.

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**R&D Systems, Inc.
1-800-343-7475**

ELISA Protocol

Solutions Required

- **Wash Buffer** - 0.05% Tween 20 in PBS, pH 7.4
- **Diluent** - 1% BSA in PBS, pH 7.4
- **Substrate Solution** - 1:1 mixture of Color Reagent A (H₂O₂) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999)
- **Stop Solution** - 1 M H₂SO₄

Plate Preparation

1. Transfer 100 µL/well of the capture antibody (diluted to the appropriate concentration in PBS, use immediately) to an ELISA plate. Seal plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette, 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 by inverting the plate and blotting it against clean paper toweling.
3. Block plates by adding 300 µL of PBS containing 1% BSA, 5% sucrose and 0.05% NaN₃ to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition. Alternatively, the blocking buffer can be aspirated after step 3 and the plates can be dried under vacuum. When sealed with desiccant, the plates can be stored at 4° - 8° C for at least 2 months.

Assay Procedure

1. Dilutions of unknowns and standards should be carried out in polypropylene tubes. Add 100 µL of sample or standards in an appropriate diluent, per well. Mix by gently tapping the plate frame for 1 minute. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 µL of the biotinylated detection antibody, diluted in the appropriate diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL streptavidin HRP (R&D Systems, Catalog # DY998, dilute according to the directions on the vial label) to each well. Cover the plate and incubate for 20 minutes at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 - 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation of Results

To calculate assay results, average the duplicate readings and subtract the zero standard optical density from the sample optical density. Create a standard curve using data reduction software capable of generating a four parameter (4P-L) curve fit. Alternatively, plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log-log paper and regression analysis may be applied to the log transformation. To determine the human BMP-4 concentrations for each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human BMP-4 concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. A standard curve should be generated for each set of samples assayed.

Limitations

It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in the above protocol may be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.

A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays. The protocol provided is for demonstration purposes only. The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range.