

Product Information

ANTI-BONE MORPHOGENETIC PROTEIN 5 (BMP-5) Developed in Goat, Affinity Isolated Antibody

Product Number **B 3805**

Product Description

Anti-Human Bone Morphogenetic Protein 5 (BMP-5) is developed in goat using a purified recombinant human bone morphogenetic protein 5 expressed in mouse NSO cells as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-BMP-5 antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Human Bone Morphogenetic Protein 5 recognizes recombinant human BMP-5 by various immuno-chemical techniques including neutralization, immunoblotting, ELISA, and immunohistochemistry. Based on ELISA, this antibody shows less than 5 % cross-reactivity with recombinant human BMP-2 and recombinant human BMP-4.

Recombinant Human BMP-5 is produced from a DNA sequence encoding a chimeric protein containing the human BMP-2 signal peptide and propeptide (amino acid residues 1 to 282) fused to the human BMP-5 mature chain (amino acid residues 323 to 454).¹ Recombinant BMP-5, a disulfide-linked homodimeric protein, consists of two 167 amino acid residue subunits with a calculated molecular mass of approximately 18 kDa. Due to glycosylation, the protein migrates as a doublet of 20 kDa and 25 kDa under reducing conditions in SDS-PAGE. BMP-5 is synthesized as a large precursor protein that is cleaved at the dibasic cleavage site (RXXR) to release the carboxy-terminal domain.

Bone Morphogenetic Proteins (BMP) are members of the TGF- β superfamily of cytokines that affect bone and cartilage formation.^{2,3,4} Similar to other TGF- β family proteins, BMPs are highly conserved across animal species. Mature BMPs are 30-38 kDa proteins that assume a TGF- β -like cysteine knot configuration. Unlike TGF- β , BMPs do not form latent complexes with their propeptide counterparts. Most BMPs are homodimers, but bioactive natural heterodimers have been reported. Recently it was found that lovostatin

(Mevinolin, Product M 2147), widely used for lowering cholesterol, also increases bone formation by turning on a gene (bmp-2) that promotes local bone formation.⁵ BMPs create an environment conducive for bone marrow development by stimulating the production of specific bone matrix proteins and altering stromal cell and osteoclast proliferation.^{6,7} In addition to stimulating ectopic bone and cartilage development, BMPs may be an important factor in the development of the viscera, with roles in cell proliferation, apoptosis, differentiation, and morphogenesis.^{2,8} BMPs also appear to be responsible for normal dorsal/ventral patterning. BMPs are found in tissues that induce bone or cartilage growth, such as demineralized bone and urinary epithelium.

Cellular responses to BMP-5 are mediated by the formation of hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors⁹ which play significant roles in BMP binding and signaling. One BMP type II receptor and two BMP type I receptors have been identified.

Reagent

Anti-human BMP-5 is supplied as 100 μ g of antiserum lyophilized from a 0.2 μ m filtered solution of phosphate buffered saline (PBS).

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline (PBS) to produce a 0.1 mg/ml stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at -20°C . Reconstituted product may be stored at 2°C to 8°C for at least one month. For prolonged storage, freeze in working aliquots at -20°C . Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

Product Profile

Anti-human BMP-5 has the ability to neutralize the biological activity of recombinant human BMP-5 on MC3T3-E1 cells.¹⁰ Recombinant human BMP-5 is

added to various concentrations of the antibody for 1 hour at 37 °C in a 96 well microplate. Following this pre-incubation, MC3T3-E1 cells are added to the mixture. The assay mixture in a total volume of 100 µl, containing antibody at concentrations of 0.01 µg/ml to 100 µg/ml, recombinant human BMP-5 at 1.5 µg/ml, L-ascorbic acid at 50 µg/ml, and cells at 5×10^4 cells/ml, is incubated at 37 °C for 4 days in a humidified CO₂ incubator. At the end of the incubation, alkaline phosphatase activity in cell lysate is measured. The Neutralization Dose₅₀ (ND₅₀) for anti-human BMP-5 is approximately 3 to 10 µg/ml in the presence of 1.5 µg/ml of recombinant human BMP-5, using the MC3T3-E1 cell line.

The ND₅₀ is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize human BMP-5 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

For immunoblotting, a working concentration of 0.1 to 0.2 µg/ml antibody is recommended. The detection limit for recombinant human BMP-5 is approximately 5 ng/lane under non-reducing and reducing conditions. For ELISAs, a working concentration of 0.5 to 1.0 µg/ml antibody is recommended. The detection limit for recombinant human BMP-5 is approximately 0.16 ng/well.

For cellular immunohistochemistry, a working concentration of at least 1 to 3 µg/ml antibody is recommended to detect human BMP-5. Cells may be fixed for 20 minutes at room temperature with freshly-prepared 1 to 2 % paraformaldehyde/PBS (pH 7.4). Three to five washes of the cells in PBS (15 minutes each) is usually required after fixation and before the addition of the primary antibody (anti-BMP-5). Labeling may be obtained by incubating the cells overnight at 4 °C with at least 1 to 3 µg/ml anti-BMP-5 antibody followed by the appropriate secondary reagents. For tissue immunohistochemistry, a working concentration of at least 5 to 10 µg/ml antibody is recommended to detect human BMP-5 on cryostat sections 5 to 15 µm thick. Dissected tissues should be

fixed by vascular perfusion with 4 % paraformaldehyde/PBS (pH 7.4) and followed by perfusion with a 10 % sucrose solution in 0.1 M phosphate buffer (pH 7.2). Note: prolonged fixation in 4 % paraformaldehyde can diminish the labeling and staining intensity of BMP-5. Cryostat sections from 5 to 15 µm are incubated with the primary antibody (anti-BMP-5) at concentrations of at least 5 to 10 µg/ml. Note: on free-floating sections, anti-BMP-5 should be diluted to at least 0.3 to 1 µg/ml.

The labeling detection system on cells and tissues can be done by either fluorescent or non-fluorescent enzymatic assays. Due to autofluorescence in neuronal tissues, the use of fluorescence detectors such as FITC or Cy3 is not recommended unless the autofluorescence is quenched (example: treating tissues after IHC staining with 1% Sudan Black in 70 % alcohol). Non-fluorescent enzymatic staining using DAB, AEC, or immunogold-silver can be used.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

Endotoxin level is < 10 ng/mg antibody as determined by the LAL (Limulus amoebocyte lysate) method.

References

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KAA 05/01

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