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Product Information

Anti-Bone Morphogenetic Protein 5
produced in goat, affinity isolated antibody

Catalog Number **B3805**

Product Description

Anti-Bone Morphogenetic Protein 5 (BMP-5) is produced in goat using a purified recombinant human bone morphogenetic protein 5, expressed in mouse NSO cells, as immunogen. Affinity isolated 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-Bone Morphogenetic Protein 5 recognizes human BMP-5 by various immunochemical techniques including neutralization, immunoblotting, and immunohistochemistry.

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 ~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, Catalog No.M2147), 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 receptors [9] which play significant roles in BMP binding and signaling. One BMP type II receptor and two BMP type I receptors have been identified.

Reagent

Supplied lyophilized from a 0.2 μm filtered solution of phosphate buffered saline with 5% trehalose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute at 0.2 mg/mL in sterile phosphate buffered saline (PBS).

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °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

Neutralization: Anti-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 1.0 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 CO_2 incubator. At the end of the incubation, alkaline phosphatase activity in cell lysate is measured.

The Neutralization $Dose_{50}$ (ND_{50}) is 6-24 µg/ml in the presence of 1.0 µg/ml of recombinant human BMP-5, using the MC3T3-E1 cell line.

The ND_{50} 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.

Immunoblotting: a working antibody concentration of 0.1 µg/ml antibody is recommended. The detection limit for recombinant human BMP-5 is ~1 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry (paraffin-embedded sections): a working antibody concentration of at least 5-15 µg/ml antibody is recommended to detect human BMP-5 on human placenta or osteosarcoma tissue sections.

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

References

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IDC,PHC 03/12-1