Product Information

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Monoclonal Anti-Bone Morphogenetic Protein 6
Clone 74219.11
produced in mouse, purified immunoglobulin

Catalog Number **B3680**

Product Description

Monoclonal Anti-Bone Morphogenetic Protein 6 (BMP-6) (mouse IgG2B isotype) is produced from a mouse hybridoma elicited from a mouse immunized with purified recombinant human BMP-6 expressed in CHO cells. The antibody is purified from the IgG fraction of ascites fluid using protein G chromatography.

Monoclonal Anti-Bone Morphogenetic Protein 6 recognizes recombinant human BMP-6 by various immunochemical techniques including capture ELISA, immunoblotting, and neutralization. By immunoblotting, this antibody shows no cross-reactivity with recombinant human (rh) BMP-2, rhBMP-4, rhBMP-5, and rhBMP-7.

BMP-6 is produced from a DNA sequence encoding the human BMP-2 signal peptide and human BMP-2 propeptide (amino acid residues 1 to 282) fused to the human BMP-6 mature subunit (amino acid residues 382 to 513) expressed in mouse myeloma NSO cells.[1] Mature human BMP-6, generated after the proteolytic removal of the signal peptide and the propeptide, is a disulfide-linked homodimeric protein, comprised of two 132 amino acid residue subunits. Each has a calculated molecular mass of approximately 15 kDa. Due to glycosylation, the recombinant protein migrates as a doublet of approximately 18 kDa and 23 kDa under reducing conditions in SDS-PAGE. Mature human and mouse BMP-6 share 96 % amino acid sequence identity.

Bone Morphogenetic Proteins are members of the TGF-β superfamily of cytokines that affect bone and cartilage formation.[2-4] 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. Lovostatin (Mevinolin, Cat. 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 are involved in embryogenesis and morphogenesis of various tissues and organs. They 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. They regulate the growth, differentiation, chemotaxis, proliferation, and apoptosis of various cell types, including mesenchymal cells, epithelial cells, hematopoietic cells, and neuronal cells.[2,8] BMPs also appear to be responsible for normal dorsal/ventral patterning and can found in tissues that induce bone or cartilage growth, such as demineralized bone and urinary epithelium.

BMP-6 is an autocrine stimulator of chondrocyte differentiation[9] and has been implicated in the development of embryonic kidney and urinary systems. It is involved in liver growth and differentiation,[10] keratinocyte differentiation,[11] and regulation of neuronal tissue development. BMP-6 expression is localized to muscle cells in the developing human fetal intestine,[12] expanding its role as a regulator of developing tissues. Cellular responses to BMP-6 are mediated by the formation of hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors,[13] which play significant roles in BMP binding and signaling. Two BMP type I receptors and one BMP type II receptor have been identified.

Reagent

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

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of sterile phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at −20 °C. Reconstituted product may be stored at 2-8 °C for up to 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: The ND_{50} of Monoclonal Anti-BMP-6 is measured by its ability to neutralize the bioactivity of rhBMP-6 in the presence of 150 ng/mL of rhBMP-6, using alkaline phosphatase production by the ATDC-5 cell line as an assay.

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-6 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Capture ELISA: this antibody can be used in a human BMP-6 ELISA in combination with biotinylated rhBMP-6 affinity purified polyclonal detection antibody. Using plates coated with 100 µL/well of the capture antibody at 4 µg/mL, in combination with 100 µL/well of the detection antibody at 200 ng/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 8 ng/mL. In this format, no cross-reactivity was observed with rhBMP-2, rhBMP-4, and rhBMP-7. Less than 0.75% cross-reactivity was observed with rhBMP-5.

Immunoblotting: a working concentration of 1-2 µg/mL is recommended. The detection limit for recombinant human BMP-6 is ~25 ng/lane under non-reducing conditions.

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

Endotoxin level is <1 EU per 1 µg of the antibody as determined by the LAL (Limulus amebocyte lysate) method.

References

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