Monoclonal Anti-human BMP-4 Antibody

ORDERING INFORMATION

Catalog Number: MAB757

Clone: 66119

Lot Number: CEO01

Size: 500 μg

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human BMP-4

Immunogen: NS0-derived rhBMP-4

Ig class: mouse IgG_{2B}

Applications: Neutralization of bioactivity
Western blot
ELISA

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, monomeric recombinant human bone morphogenetic protein 4 (rhBMP-4). The IgG fraction of ascites fluid was purified by Protein G affinity chromatography.

Formulation
Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level
< 0.1 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 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 neutralize human BMP-4 bioactivity. Based on direct ELISA and western blot results, this antibody shows approximately 5% cross-reactivity with rhBMP-2, rmBMP RIA and rmBMP RIB.

Neutralization of Human BMP-4 Bioactivity
The exact concentration of antibody required to neutralize human BMP-4 activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization Dose$_{50}$ (ND_{50})** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

The ND_{50} for this lot of anti-human BMP-4 antibody was determined to be approximately 1 - 3 μg/mL in the presence of 500 ng/mL of rhBMP-4, using the C2C12 cell line. The specific conditions are described in the figure legends.

Additional Applications
Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human BMP-4. The detection limit for rhBMP-4 is approximately 6 ng/well.

Western blot - This antibody can be used at 1 - 2 μg/mL with the appropriate secondary reagents to detect human BMP-4. The detection limit for rhBMP-4 is approximately 50 ng/lane under non-reducing and reducing conditions.

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

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

Figure 1

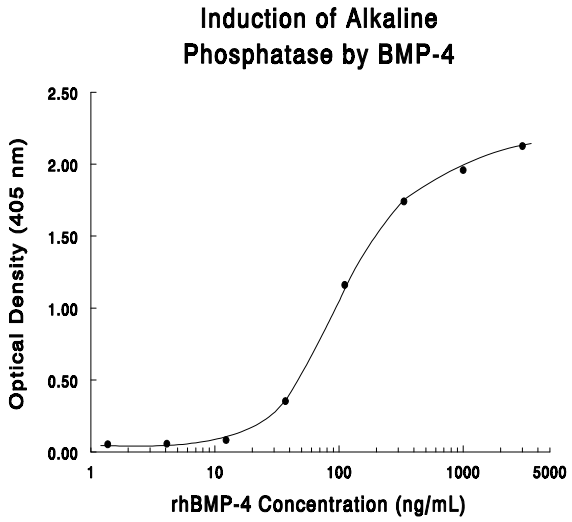

Figure 2

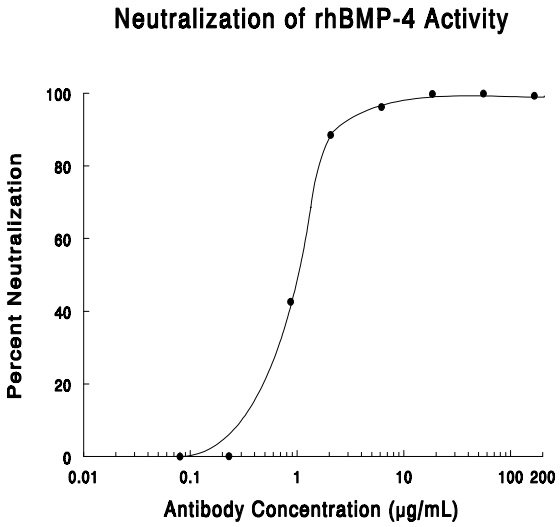

Figure 1
Human BMP-4 induces alkaline phosphatse production by C2C12 myoblasts (Katagiri, T. *et al*., 1994, J. Cell Biol. **127**:1755 - 1766). The ED_{50} for this effect is typically 50 - 200 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of human BMP-4, rhBMP-4 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well plate. Following this preincubation period, C2C12 cells were added. The assay mixture in a total volume of 100 µL/well, containing antibody at the concentrations indicated, rhBMP-4 at 500 ng/mL, heparin at 2 µg/mL and cells at 3 x 10^4/mL, was incubated at 37° C for 3 days in a humidified CO_2 incubator. At the end of incubation, alkaline phosphatase activity in cell lysate was tested. The ND_{50} of this antibody under these conditions is approximately 1 - 3 µg/mL.