



Anti-human BMP-6 Antibody

ORDERING INFORMATION

Catalog Number: AF507

Lot Number: CXL04

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human BMP-6

Immunogen: *E. coli*-derived rhBMP-6

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Immunohistochemistry
Direct ELISA

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human bone morphogenetic protein 6 (rhBMP-6). BMP-6 specific IgG was purified by human BMP-6 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 0.1 mg/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 has been selected for its ability to neutralize rhBMP-6 bioactivity. In direct ELISAs, this antibody shows approximately 10% cross-reactivity with rhBMP-7 and no cross-reactivity with rhBMP-2 and rhBMP-4.

Neutralization of Human BMP-6 Bioactivity

The exact concentration of antibody required to neutralize rhBMP-6 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 Dose₅₀ (ND₅₀)** 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 ND₅₀ for this lot of anti-human BMP-6 antibody was determined to be approximately 5 - 20 µg/mL in the presence of 300 ng/mL of rhBMP-6, using the ATDC5 cell line. The specific conditions are described in the figure legends.

Additional Applications

Western blot - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect human BMP-6. The detection limit for rhBMP-6 is approximately 10 ng/lane under both non-reducing and reducing conditions.

Immunohistochemistry - This antibody will detect BMP-6 in cells or tissues. The working dilution is 15 µg/mL. For chromogenic detection of labeling, it is recommended to use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

Direct ELISAs - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human BMP-6. The detection limit for rhBMP-6 is approximately 0.3 ng/well.

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

For immunohistochemistry images, please refer to our website at <http://www.RnDSystems.com/ihc>

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Figure 1

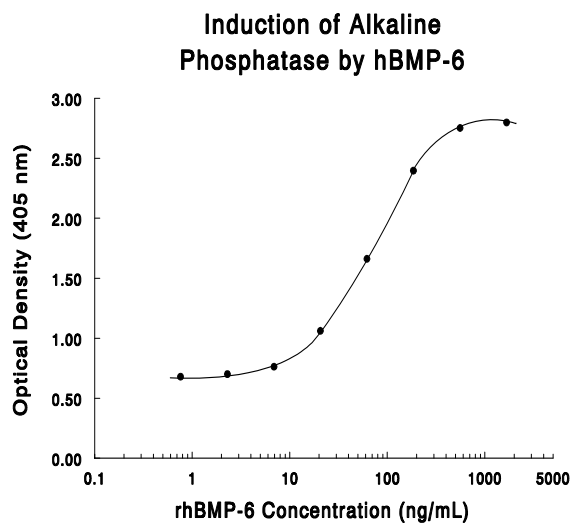


Figure 2

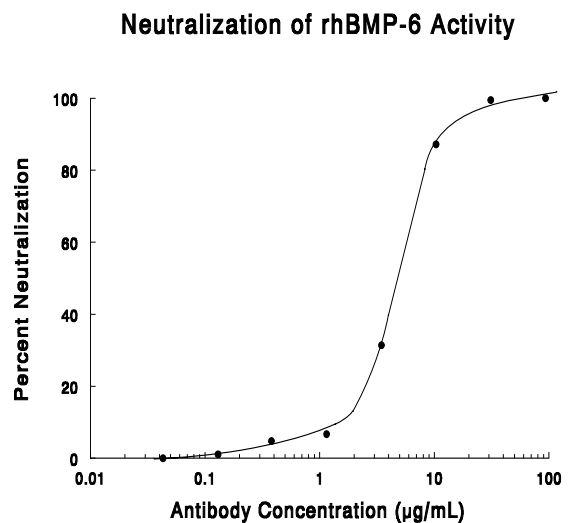


Figure 1

Human BMP-6 induces alkaline phosphatase expression by a mouse chondrogenic cell line, ATDC5 (Asahina, I. *et al.*, 1996, *Exp. Cell Res.* **222**:38 - 47). The ED_{50} for this effect is typically 50 - 150 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of human BMP-6, rhBMP-6 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well plate. Following this preincubation period, ATDC5 cells were added. The assay mixture in a total volume of 100 µL/well, containing antibody at the concentrations indicated, rhBMP-6 at 300 ng/mL, L-ascorbic acid at 50 µg/mL and cells at 1×10^5 cells/mL, was incubated at 37° C for 3 days in a humidified CO₂ incubator. At the end of the incubation, alkaline phosphatase activity in the cell lysate was tested. The ND_{50} of this antibody, under these conditions, is approximately 5 - 20 µg/mL.