



## ***Anti-human BMP-4 Antibody***

### **ORDERING INFORMATION**

**Catalog Number:** AF757

**Lot Number:** DND02

**Size:** 100 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 Type:** goat IgG

**Applications:** Neutralization of bioactivity  
Western blot  
ELISA

### ***Preparation***

Produced in goats immunized with purified, NS0-derived, recombinant human bone morphogenetic protein 4 (rhBMP-4). Human BMP-4 specific IgG was purified by human BMP-4 affinity chromatography.

### ***Formulation***

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

### ***Endotoxin Level***

< 0.3 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 human BMP-4 bioactivity.

### ***Neutralization of Human BMP-4 Bioactivity***

The exact concentration of antibody required to neutralize rhBMP-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<sub>50</sub> (ND<sub>50</sub>)** 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<sub>50</sub> for this lot of anti-human BMP-4 antibody was determined to be approximately 0.5 - 2 µg/mL in the presence of 30 ng/mL of rhBMP-4, using the ATDC-5 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-4. The detection limit for rhBMP-4 is approximately 5 ng/lane under non-reducing and reducing conditions.

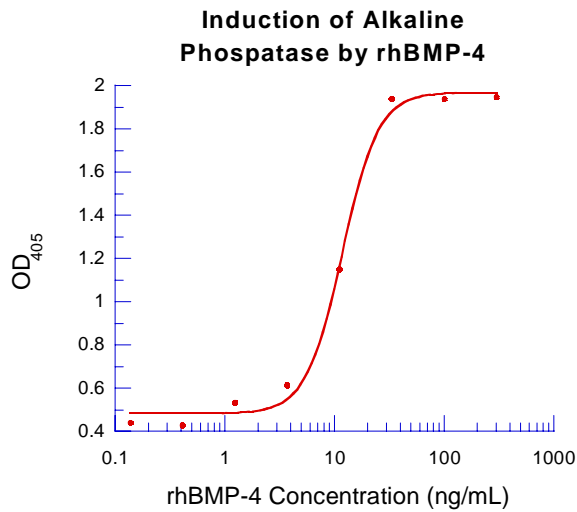
**Direct ELISA** - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human BMP-4. The detection limit for rhBMP-4 is approximately 0.6 ng/well. In this format, this antibody shows approximately 35% cross-reactivity with rhBMP-2 and 10% cross-reactivity with rhBMP-5 and rhBMP-6.

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

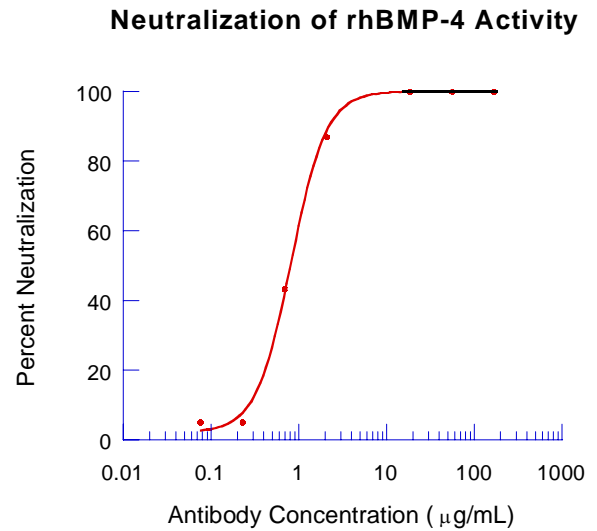
FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Figure 1**



**Figure 2**



**Figure 1**

Human BMP-4 induces alkaline phosphatase production by ATDC-5 cells (Nakamura, K. *et al.*, 1999, *Exp. Cell Res.* **250**:351). The ED<sub>50</sub> for this effect is typically 10 - 30 ng/mL.

**Figure 2**

To measure the ability of the antibody to neutralize the bioactivity of 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, ATDC-5 cells were added. The assay mixture in a total volume of 100 µL/well, containing antibody at the concentrations indicated, rhBMP-4 at 30 ng/mL, heparin at 2 µg/mL and cells at 1 x 10<sup>5</sup> cells/mL, was incubated at 37° C for 3 days in a humidified CO<sub>2</sub> incubator. At the end of incubation, alkaline phosphatase activity in the cell lysate was determined. The ND<sub>50</sub> of this antibody under these conditions is approximately 0.5 - 2 µg/mL.