Anti-porcine GM-CSF Antibody

**Preparation**
Produced in goats immunized with purified, *E. coli*-derived, recombinant porcine granulocyte macrophage colony stimulating factor (rpGM-CSF). GM-CSF specific IgG was purified by porcine GM-CSF affinity chromatography.

**Formulation**
Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS).

**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 the bioactivity of rpGM-CSF. In direct ELISAs, this antibody shows approximately 25% cross-reactivity with rrGM-CSF, 15% cross-reactivity with rhGM-CSF and 5% cross-reactivity with rmGM-CSF.

**Neutralization of Porcine GM-CSF Bioactivity**
The exact concentration of antibody required to neutralize rpGM-CSF 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-porcine GM-CSF antibody was determined to be approximately 0.2 - 1.0 µg/mL in the presence of 10 ng/mL of rpGM-CSF, using the TF-1 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 porcine GM-CSF. The detection limit for rpGM-CSF is approximately 1 ng/lane under non-reducing and reducing conditions.

ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect porcine GM-CSF. The detection limit for rpGM-CSF is approximately 0.5 ng/well.

Optimal dilutions should be determined by each laboratory for each application.
**Figure 1**
Porcine GM-CSF stimulates the \(^3\)H-thymidine incorporation by TF-1 cells in a dose-dependent manner (Kitamura, T. *et al.*, 1989, *J. Cell Physiol.* 140(2):323 - 333). The ED\(_{50}\) for this effect is typically 2 - 8 ng/mL.

**Figure 2**
To measure the ability of the antibody to neutralize the bioactivity of rpGM-CSF on human TF-1 cells, rpGM-CSF was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well microplate. Following this preincubation period, TF-1 cells were added. The assay mixture, in a total volume of 100 µL, containing antibody at the concentrations indicated, rpGM-CSF at 10 ng/mL and cells at 1 x 10\(^5\) cells/mL, was incubated at 37° C for 48 hours in a humidified CO\(_2\) incubator. \(^3\)H-thymidine was added during the final 4 hours of incubation. The cells were harvested onto glass fiber filters and the \(^3\)H-thymidine incorporated into DNA was determined. The ND\(_{50}\) for this antibody is approximately 0.2 - 1.0 µg/mL.