Anti-feline GM-CSF Antibody

ORDERING INFORMATION

Catalog Number: AF987

Lot Number: GAC01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: feline GM-CSF

Immunogen: *E. coli*-derived rfeGM-CSF

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant feline granulocyte macrophage colony stimulating factor (rfeGM-CSF). Feline GM-CSF specific IgG was purified by feline GM-CSF 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 rfeGM-CSF bioactivity.

Neutralization of Feline GM-CSF Bioactivity

The exact concentration of antibody required to neutralize rfeGM-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.

As shown in figures 1 and 2 on the next page, the ND$_{50}$ for this lot of anti-feline antibody was determined to be approximately 0.5 - 2 μg/mL in the presence of 50 ng/mL of rfeGM-CSF, using the TF-1 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 feline and porcine GM-CSF. The detection limit for rfeGM-CSF and rpGM-CSF is approximately 1.2 ng/well. In this format, this antibody shows approximately 40% cross-reactivity with rmGM-CSF, rrGM-CSF and rhGM-CSF.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect feline and porcine GM-CSF. The detection limit for rfeGM-CSF and rpGM-CSF is approximately 5 ng/lane under non-reducing and reducing conditions.

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

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Figure 1

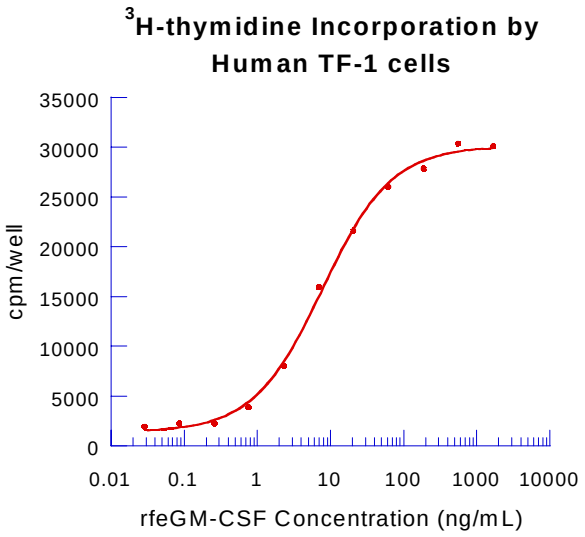

Figure 2

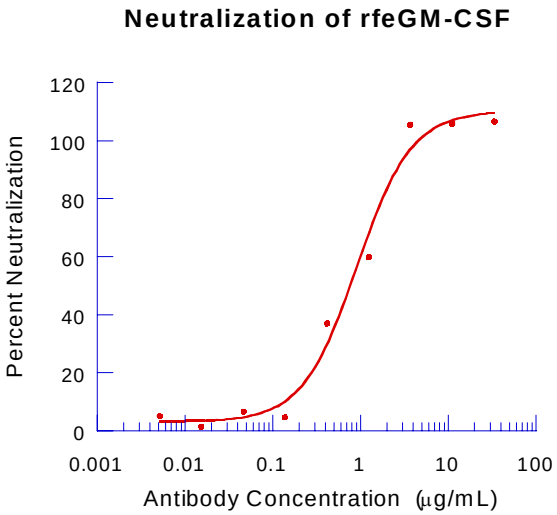

Figure 1
Feline GM-CSF stimulates the ^{3}H-thymidine incorporation by human TF-1 cells in a dose dependent manner (Kitamura, T. *et al.*, 1989, J. Cell Physiol. **140**(2):323 - 333). The ED_{50} for this effect is typically 2 - 8 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of rfeGM-CSF on human TF-1 cells, rfeGM-CSF was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96-well plate. Following this preincubation period, TF-1 cells were added. The assay mixture in a total volume of 100 µL/well, containing antibody at the concentrations indicated, rfeGM-CSF at 50 ng/mL and cells at 1 x 10^{5} cells/mL, was incubated at 37° C for 72 hours in a humidified CO_2 incubator. ^{3}H-thymidine was added during the final 4 hours of incubation. The cells were subsequently harvested onto glass fiber filters and the ^{3}H-thymidine incorporated into DNA was determined. The ND_{50} of the antibody under these conditions is approximately 0.5 - 2 µg/mL.