ANTI-GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF), RAT, Affinity Isolated Antibody
Product Number G0907

Product Description
Anti-Rat Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is developed in goat using purified recombinant rat GM-CSF, expressed in *E. coli*, as immunogen. The antibody is purified using rat GM-CSF affinity chromatography.

Anti-Rat GM-CSF may be used to neutralize the bioactivity of recombinant rat GM-CSF. The antibody may also be used for immunoblotting and ELISA. By ELISA and immunoblotting, the antibody shows 30% cross-reactivity with recombinant mouse GM-CSF, and 10% cross-reactivity with recombinant human GM-CSF.

Four distinct colony-stimulating factors (CSFs) that promote survival, proliferation and differentiation of bone marrow precursor cells are well characterized: granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), and interleukin-3 (IL-3, Multi-CSF).1,2 Both GM-CSF and IL-3 are multipotential growth factors, stimulating proliferation of progenitor cells from more than one hematopoietic lineage. In contrast, G-CSF and M-CSF are lineage-restricted hematopoietic growth factors, stimulating the final mitotic divisions and the terminal cellular maturation of partially differentiated hematopoietic progenitors.

GM-CSF induces myeloid progenitor cells from bone marrow to form colonies containing macrophages and granulocytes in a semisolid media. It acts upon mature macrophages, eosinophils and neutrophils to stimulate various functional activities.2 GM-CSF is an acidic glycoprotein (18-22 kDa in human3 and 23 kDa in mouse4) which binds to high affinity receptors on GM-CSF sensitive cells. Although human and mouse GM-CSF share 54% amino acid sequence homology, their biological actions are species-specific.4 The actions of GM-CSF and the binding of GM-CSF to its receptor are modulated by other growth factors and additional forms of CSF.5 Human GM-CSF is presently undergoing clinical trials for therapy of several diseases and syndromes.6

Reagents
Anti-Rat GM-CSF is supplied lyophilized from a 0.2 µm filtered solution of phosphate buffered saline. Endotoxin level is < 10 ng per mg antibody as determined by the LAL method.

Preparation Instructions
To one vial of lyophilized powder, add 1 ml of 0.2 µm-filtered PBS to produce a 0.1 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability
Prior to reconstitution, store at −20 °C. Reconstituted product may be stored at 2-8 °C for at least one month. For prolonged storage, freeze in working aliquots at −20 °C. Avoid repeated freezing and thawing.

Procedure
Neutralization of Bioactivity
To measure the ability of the antibody to neutralize the bioactivity of rat GM-CSF, recombinant rat GM-CSF was incubated with various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following preincubation, DA3 cells were added. The assay mixture in a total volume of 100 µl per well, containing antibody at concentrations of 0.01 µg/ml-200 µg/ml, recombinant rat GM-CSF at 0.5 ng/ml, and cells at 1x10⁵ cells/ml were incubated at 37 °C for 24 hours in a humidified CO₂ incubator. Tritiated-thymidine was added during the final 4 hours. Cells were harvested and three-H-thymidine incorporation was measured.7 The ND₅₀ is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.
Product Profile
For neutralization, a working concentration of 0.3-0.6 µg/ml of Anti-Rat GM-CSF will neutralize 50% of the bioactivity due to 0.5 ng/ml recombinant rat GM-CSF using DA3 cells.

For indirect ELISA, a working concentration of 0.5-1.0 µg/ml is determined to detect a limit of ~0.6 ng/well of recombinant rat GM-CSF.
For indirect immunoblotting, a working concentration of 0.1-0.2 µg/ml is determined using rat GM-CSF at 1 ng/lane under non-reducing and reducing conditions.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References