



## ANTI-MACROPHAGE COLONY STIMULATING FACTOR (M-CSF), MOUSE

Developed in Goat, Affinity Isolated Antibody

Product Number **M9182**

### Product Description

Anti-Mouse Macrophage Colony Stimulating Factor (M-CSF) is developed in goat using purified recombinant mouse M-CSF, expressed in *E. coli*, as immunogen. The antibody is purified using mouse M-CSF affinity chromatography.

Anti-Mouse M-CSF may be used to neutralize the bioactivity of recombinant mouse M-CSF. The antibody may also be used for immunoblotting and ELISA. By ELISA and immunoblotting, the antibody shows approximately 5% cross-reactivity with recombinant human M-CSF.

Anti-Mouse M-CSF may be used for neutralization, ELISA and immunoblotting.

Four distinct colony-stimulating factors (CSFs) that promote survival, proliferation and differentiation of bone marrow precursor cells have been well characterized: granulocyte macrophage-CSF (GM-CSF), granulocyte-CSF (G-CSF), macrophage-CSF (M-CSF), and Interleukin-3 (IL-3, Multi-CSF).<sup>1,2</sup> 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 final mitotic divisions and the terminal cellular maturation of the partially differentiated hematopoietic progenitors.

Macrophage CSF, also known as CSF-1, is produced by monocytes, fibroblasts and endothelial cells. It stimulates the formation of macrophage colonies,<sup>3</sup> enhances antibody-dependent, cell-mediated cytotoxicity by monocytes and macrophages,<sup>4</sup> and inhibits bone resorption by osteoclasts.<sup>5</sup> Natural human M-CSF is a dimeric glycoprotein of 70-90 kD molecular weight, existing in multiple glycosylation forms.<sup>6</sup> It binds to a 165 kD glycoprotein of the receptor tyrosine kinase subclass III,<sup>7</sup> a family that includes the receptors for platelet derived growth factor (PDGF) and stem cell factor (SCF).

## Product Information

### Reagents

Anti-Mouse M-CSF is supplied lyophilized from a 0.2  $\mu$ m 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  $\mu$ m 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  $-20^{\circ}\text{C}$ . Reconstituted product may be stored at  $2-8^{\circ}\text{C}$  for at least one month. For prolonged storage, freeze in working aliquots at  $-20^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

### Procedure

#### Neutralization of Bioactivity

To measure the ability of the antibody to neutralize the bioactivity of mouse M-CSF, recombinant mouse M-CSF was incubated with various concentrations of the antibody for 1 hour at  $37^{\circ}\text{C}$  in a 96 well plate. Following preincubation, M-NFS-60 cells were added. The assay mixture in a total volume of 100  $\mu$ l per well, containing antibody at concentrations of 0.001-1  $\mu$ g/ml, recombinant mouse M-CSF at 2.5 ng/ml, and cells at  $1 \times 10^5$  cells/ml were incubated at  $37^{\circ}\text{C}$  for 24 hours in a humidified  $\text{CO}_2$  incubator. Tritiated-thymidine was added during the final 4 hours. Cells were harvested and  $^3\text{H}$ -thymidine incorporation was measured.<sup>8</sup> The  $\text{ND}_{50}$  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 0.015-0.035  $\mu$ g/ml of Anti-Mouse M-CSF will neutralize 50% of the bioactivity due to 2.5 ng/ml recombinant mouse M-CSF using M-NFS-60 cells.

For indirect ELISA, a working concentration of 0.5-1.0 µg/ml is determined to detect a limit of ~0.16 ng/well of recombinant mouse M-CSF.

For indirect immunoblotting, a working concentration of 0.1-0.2 µg/ml is determined using mouse M-CSF at 5 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

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