

## Product Information

### Anti-Granulocyte Colony Stimulating Factor

produced in goat, affinity isolated antibody

Catalog Number **G4921**

#### Product Description

Anti-Granulocyte Colony Stimulating Factor is produced in goat using as immunogen purified, recombinant mouse granulocyte colony stimulating factor (G-CSF), expressed in *E. coli* (Gene ID: 1440). G-CSF specific IgG was purified by mouse G-CSF affinity chromatography.

Anti-Granulocyte Colony Stimulating Factor recognizes mouse G-CSF by various immunochemical techniques including neutralization and immunoblotting. The antibody has been selected for its ability to neutralize the biological activity of recombinant mouse G-CSF. It will also neutralize the biological activity of recombinant human G-CSF at a 30-60 fold higher IgG concentration.

Four distinct colony-stimulating factors (CSFs) promoting 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> G-CSF and M-CSF are lineage-restricted hematopoietic growth factors, stimulating final mitotic divisions and terminal cellular maturation of partially differentiated hematopoietic progenitors.

Granulocyte colony stimulating factor is produced by: macrophages activated by endotoxin (LPS), monocytes activated by TNF $\alpha$  with INF $\gamma$ , fibroblasts and endothelial cells activated by IL-1 or TNF- $\alpha$ , and bone marrow stromal cells activated by IL-1 or LPS.<sup>3,4</sup> In addition, various carcinoma cell lines and myeloblastic leukemia cells can express G-CSF constitutively. G-CSF stimulates granulocyte colony formation, activates neutrophils and other mature granulocytes, and promotes differentiation of certain myeloid leukemic cells. G-CSF acts on mature neutrophils to enhance their survival and to stimulate their tumoricidal activity. It will also synergize with IL-3 and shorten the G<sub>0</sub> period of early hematopoietic progenitors. G-CSF has important roles in defense against infection, in inflammation and repair processes, and in maintenance of steady state hematopoiesis.

#### Reagent

Supplied lyophilized from a 0.2  $\mu$ m filtered solution of phosphate buffered saline containing 5% trehalose.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

To one vial of lyophilized powder, add 1 mL of sterile PBS to produce a 0.1 mg/mL stock solution.

#### Storage/Stability

Prior to reconstitution, store at -20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

#### Product Profile

##### Neutralization of Bioactivity:

To measure the ability of this antibody to neutralize the bioactivity of mouse G-CSF on mouse NFS-60 cells, recombinant mouse G-CSF was incubated with various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following this preincubation period, NFS-60 (mouse myeloblastic) cells were added. The assay mixture in a total volume of 200  $\mu$ L per well, containing antibody at concentrations of 0.0001  $\mu$ g/mL to 1.0  $\mu$ g/mL, recombinant mouse G-CSF at 0.125 ng/mL, and cells at 5x10<sup>4</sup> cells/mL was incubated at 37 °C for 24 hours in a humidified CO<sub>2</sub> incubator. <sup>3</sup>H-thymidine is added during the final four hours. Cells are harvested and <sup>3</sup>H-thymidine incorporated into DNA was determined.<sup>5</sup>

The ND<sub>50</sub> 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.

**Note:** The exact concentration of antibody required to neutralize mouse G-CSF activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working concentration of 0.1-0.2 µg/mL is recommended. The detection limit for rmG-CSF is ~5 ng/lane under non-reducing and reducing conditions.

**Note:** In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

Endotoxin level is <0.1 EU/µg of antibody as determined by the LAL method.

## References

1. Nagata, S., Granulocyte colony-stimulating factor (G-CSF), in *Guidebook to Cytokines and Their Receptors*, Nicola, N., ed., Oxford Press (New York, NY: 1994), pp. 158-160.
2. Murakami, H., and Nagata, S., Granulocyte colony stimulating factor, in *The Cytokine Handbook*, 3<sup>rd</sup> Edition, Thomson, A.W., ed., Academic Press (San Diego, CA: 1998), pp. 671-688.
3. Nagata, S. et al., Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature*, **319**, 415 (1986).
4. Souza, L., et al., Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science*, **232**, 61 (1986).
5. Shirafuji, N., et al., A new bioassay for human granulocyte colony-stimulating factor (hG-CSF) using murine myeloblastic NFS-60 cells as targets and estimation of its levels in sera from normal healthy persons and patients with infectious and hematological disorders. *Exp. Hematol.*, **17**, 116-119 (1989).

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