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# Product Information

**Anti-Granulocyte Colony Stimulating Factor**
produced in goat, affinity isolated antibody

Catalog Number **G5046**

## Product Description

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

Anti-Granulocyte Colony Stimulating Factor recognizes human 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 human G-CSF. Based on immunoblotting, the antibody shows less than 40% cross-reactivity with recombinant mouse G-CSF.

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).[1, 2] 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.[3, 4] 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 tumorcidal activity. It will also synergize with IL-3 and shorten the $G_0$ 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 μm filtered solution of phosphate buffered saline containing 5% trehalose.

## 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 freezers.

## Product Profile

Neutralization of Bioactivity:
To measure the ability of this antibody to neutralize the bioactivity of human G-CSF on mouse NFS-60 cells, recombinant human 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 100 μL per well, containing antibody at concentrations of 0.0001 μg/mL to 10 μg/mL, recombinant human G-CSF at 0.125 ng/mL, and cells at $1x10^5$ cells/mL was incubated at 37 °C for 48 hours in a humidified $CO_2$ incubator and pulsed with resazurin for the final 16-20 hours. The fluorescence was then read in a microplate plate reader set at 544/590 nm.

The $ND_{50}$ 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.

The exact concentration of antibody required to neutralize human 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 recombinant human G-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 antibody as determined by the LAL (Limulus amebocyte lysate) 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, 3rd 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).


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