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Product Information

Monoclonal Anti-Granulocyte Colony Stimulating Factor
Clone 67604
produced in mouse, purified immunoglobulin

Catalog Number **G5421**

Product Description

Monoclonal Anti-Granulocyte Colony Stimulating Factor (rat IgG1 isotype) is produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells from a rat immunized with purified, recombinant mouse granulocyte colony stimulating factor (G-CSF), expressed in *E. coli* (Gene ID: 1440). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Monoclonal Anti-Granulocyte Colony Stimulating Factor recognizes mouse G-CSF by various immunochemical techniques including neutralization, immunoblotting, and capture ELISA. The antibody neutralizes the biological activity of recombinant mouse G-CSF. When used as a capture antibody in sandwich ELISAs, the antibody shows less than 0.06% cross-reactivity with recombinant human G-CSF, recombinant human CNTF, recombinant mouse IL-6, recombinant mouse LIF, and recombinant mouse OSM.

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 TNFα with INFγ, fibroblasts and endothelial cells activated by IL-1 or TNF-α, 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.

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 0.5 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, recombinant mouse G-CSF is 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 are added. The assay mixture in a total volume of 200 μL per well, containing antibody at concentrations of 0.0001 μg/mL to 10 μg/mL, recombinant mouse G-CSF at 0.125 ng/mL, and cells at ~$5x10^4$ cells/mL are incubated at 37 °C for 24 hours in a humidified CO_2 incubator. ^{3}H-thymidine is added during the final four hours. Cells are harvested and ^{3}H-thymidine incorporation is measured.[6]

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.

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.

ELISA capture: The antibody can be used as a capture antibody in a mouse G-CSF sandwich immunoassay in combination with a biotinylated mouse G-CSF detection antibody and recombinant mouse G-CSF as the standard. The suggested coating concentration range is 2-8 μg/mL and should be titrated to determine the optimal concentration.

Immunoblotting: a working concentration of 1-2 μg/mL is recommended. The detection limit for rmG-CSF is ~50 ng/lane under non-reducing and reducing conditions. Chemiluminescent detection will increase the sensitivity by 5 to 50 fold.

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 EUμg antibody as determined by the LAL (Limulus amebocyte lysate) method.

References

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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).
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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