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Product Information

Anti-Granulocyte Colony Stimulating Factor
produced in goat, IgG fraction of antiserum

Catalog Number **G5296**

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). Total IgG was purified by protein G 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 not neutralize the biological activity of recombinant human G-CSF. Based on immunoblotting, the antibody shows less than 20% cross-reactivity with recombinant human 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 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 grasnulocytes, 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

Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the 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 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 rmG-CSF on mouse NFS-60 cells, rmG-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 μL per well, containing antibody at concentrations of 0.01–100 μg/mL, rmG-CSF at 0.125 ng/mL, and cells at 5×10^4 cells/mL was 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 incorporated into DNA was determined.[5]

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 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 1–2 μg/mL is recommended. The detection limit for rmG-CSF is ~5 ng/lane and 2 ng/lane under non-reducing and reducing conditions, respectively.

Note: In order to obtain the best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

Endotoxin level is <10 ng/mg 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).
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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