Product Information

Monoclonal Anti-Granulocyte Colony Stimulating Factor, clone 3316

Produced in mouse, Purified immunoglobulin

G1029

Product Description

Monoclonal Anti-Granulocyte Colony Stimulating Factor (mouse IgG1 isotype) is produced from a mouse hybridoma elicited from a mouse immunized with purified recombinant human granulocyte colony stimulating factor (G-CSF), expressed in *E.coli* (Gene ID: 1440). The antibody is purified from tissue culture supernatant using Protein G.

Monoclonal Anti-Granulocyte Colony Stimulating Factor recognizes recombinant human G-CSF by various immunochemical techniques including neutralization and capture ELISA.

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.

In humans, two distinct cDNA clones for G-CSF, encoding 207 and 204 amino acid precursor proteins, have been isolated.[3,4] Both proteins have a 30 amino acid signal peptide and identical amino acid sequences except for a three amino acid insertion (deletion) at the 35th amino acid residue from the N-terminus of the mature protein. Natural G-CSF is a glycoprotein of 177 amino acids and a molecular mass of ~18.8 kDa. Human and mouse G-CSF share ~73 % amino acid sequence homology and show biological cross-reactivity.

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 leukemia 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_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 phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody.

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 human G-CSF, recombinant human 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 0.001–10 µg/mL, recombinant human G-CSF at 0.125 ng/mL, and cells at ~5 x 10^4 cells/mL are incubated at 37 °C for 24-hours in a humidified CO_2 incubator. Tritiated-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.

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.

Capture ELISA

Use 1 µg/mL of this antibody as the capture antibody. In the ELISA capture assay, plates are coated with 100 µL/well of the capture antibody at 1 µg/mL in combination with 100 µL/well of a detection antibody (affinity-purified biotinylated polyclonal anti-human G-CSF antibody) at 100–200 ng/mL. An ELISA range of 15.6–1,000 pg/mL can be obtained.

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 per 1 µg of the 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 Handbood, 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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