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Product Information

Monoclonal Anti-Granulotyte Colony Stimulating Factor
Clone 67604.111
Purified Rat Immunoglobulin

Catalog Number **G0540**
Storage Temperature –20 °C

Product Description

Monoclonal Anti-Granulocyte Colony Stimulating Factor (G-CSF) (rat IgG1 isotype) is derived from a hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a rat immunized with purified recombinant mouse G-CSF, expressed in *E. coli*. The antibody is purified from tissue culture supernatant using protein G.

Monoclonal Anti-G-CSF may be used to neutralize the bioactivity of recombinant mouse G-CSF. The antibody may also be used for immunoblotting and ELISA. By ELISA and immunoblotting, the antibody shows no cross-reactivity with recombinant human G-CSF.

Monoclonal Anti-G-CSF may be used for neutralization, ELISA, and immunoblotting.

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] Both GM-CSF and IL-3 are multipotential growth factors, stimulating proliferation of progenitor cells from more than one hematopoietic lineage. In contrast, G-CSF and M-CSF are lineage-restricted hematopoietic growth factors, stimulating final mitotic divisions and the terminal cellular maturation of the partially differentiated hematopoietic progenitors.

Granulocyte CSF is produced by monocytes and fibroblasts.[3,4] It stimulates granulocyte colony formation, activates neutrophils and mature granulocytes, and promotes differentiation of certain myeloid leukemic cells. Natural G-CSF is a glycoprotein of 177 amino acids and a molecular mass of 19 kDa.[5] Human and murine G-CSF have ~75% homology and show biological cross-reactivity.

Reagent

Monoclonal Anti-G-CSF is supplied lyophilized from a 0.2 μm filtered solution of phosphate buffered saline. Endotoxin level is <10 ng per mg antibody as determined by the LAL method.

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 0.2 μm filtered PBS to produce a 0.5 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2–8 °C for at least one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing.

Procedure

Neutralization of Bioactivity

To measure the ability of the antibody to neutralize the bioactivity of mouse G-CSF, 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 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, recombinant mouse G-CSF at 0.125 ng/ml, and cells at 5×10^4 cells/ml were incubated at 37 °C for 24 hours in a humidified CO_2 incubator. Tritiated-thymidine was added during the final 4 hours. Cells were harvested and ^{3}H-thymidine incorporation was 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.

Product Profile

For neutralization, a working concentration of 0.01–0.03 µg/ml of Monoclonal Anti-G-CSF will neutralize 50% of the bioactivity due to 0.125 ng/ml recombinant mouse G-CSF using NSF-60 cells.

For indirect ELISA, a working concentration of 0.5–1.0 µg/ml is determined to detect a limit of ~1.6 ng/well of recombinant mouse G-CSF.

For indirect immunoblotting, a working concentration of 1–2 µg/ml is determined using mouse G-CSF at 50 ng/lane under non-reducing and reducing conditions.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

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3. Metcalf, D., Cell, **43**, 5 (1985).
4. Groopman, J., Cell, **50**, 5 (1987).
5. Souza, L., et al., Science, **232**, 61 (1986).
6. Shirafuji, N., et al., Exp. Hematol., **17**, 116 (1989).

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