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Product Information

Monoclonal Anti-Interferon-γ, clone 25723
produced in mouse, purified immunoglobulin

Catalog Number **I9284**

Product Description

Monoclonal Anti-Interferon-γ (IFN-γ) (mouse IgG2B isotype) is purified from a hybridoma produced by the fusion of mouse myeloma cells and B cells from mice immunized with recombinant human Interferon-γ (GeneID 3458) expressed and purified from *Eschericia coli*. The antibody is purified by Protein G affinity chromatography.

Monoclonal Anti-Interferon-γ recognizes human Interferon-γ. Applications include immuno-histochemistry, flow cytometry and neutralization. This antibody was selected for its ability to neutralize the bioactivity of recombinant human IFN-γ. This antibody will not neutralize the activity of rat or mouse IFN-γ.

Mature IFN-γ is a homodimeric cytokine produced primarily by T-lymphocytes and natural killer cells. IFN-γ has many immunoregulatory and proinflammatory activities. It binds to IFN-γ receptors present on virtually all cell types. IFN-γ exerts a variety of biological effects including antiviral activity,[1] inhibition of cell or tumor growth,[2,3] and promotion of differentiation of B cells into immunoglobulin-producing cells.[4,5] In addition to antiviral activity, human IFN-γ is a potent modulator of immune response and modifies cellular processes.[6] IFN-γ is classified as immune interferon.[6] It functions as an activating factor to prime macrophages (MAF) for non-specific tumoricidal activity[7] and activates monocytes to exert enhanced cytotoxicity against tumor cells.[8] IFN-γ acts as a signal for major histocompatibility antigen expression[9] and boosts cytotoxicity of natural killer cells and stimulates T cell cytotoxicity. The species specificity of IFN-γ resides in the interaction of IFN-γ with its receptor.[10] Human IFN-γ does not bind specifically to mouse, hamster or bovine cells.[10]

Reagent

Supplied lyophilized from a 0.2 μm filtered solution of phosphate buffered saline with 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 0.2 μm filtered phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be necessary 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 up to one month. For extended storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended.

Neutralization

To measure the ability of the antibody to neutralize the bioactivity of recombinant human IFN-γ on HeLa cells, IFN-γ was added to various concentrations of the antibody. The antigen-antibody mixture was added to confluent cultures of HeLa cells in a 96 well plate. The assay mixture in a total volume of 100 µL, containing antibody at 0.001-30 µg/mL, recombinant human IFN-γ at 50 units/mL (5 ng/mL), was incubated at 37 °C for 20-24 hours in a humidified CO_2 incubator. At the end of this incubation period, medium was aspirated from all wells and an appropriate titrated amount of Encephalomyocarditis virus (EMCV) in prewarmed culture medium was added to each test well. After another additional 20-24 hour incubation period, the cells were fixed, stained and scored for cytopathic effect by measurement of optical densities in a microplate reader at 540 nm.

The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

Product Profile

Immunohistochemistry: a working concentration of 2-5 µg/mL is recommended to label human IFN-γ in activated PBLs.

Flow cytometry: for intracellular staining to detect human IFN-γ, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin. Dilute this antibody to 25 µg/mL and add 10 µL of this solution to 1-5 x 10^5 cells in a total reaction volume not exceeding 200 µL. Following a 30 minute incubation, cell should be washed with 0.1% saponin prior to adding 10 µL of a 25 µg/mL stock solution of a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome. Cells should be washed for a final time in 0.1% saponin prior to flow cytometric analysis.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

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