3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Interferon-γ
produced in goat, affinity isolated antibody

Catalog Number **I5027**

Synonym: Anti-IFN-γ

Product Description

Anti-Interferon-γ is produced in goat using as immunogen a purified recombinant rat interferon-γ expressed in *E. coli*. Affinity isolated antibody is obtained from Anti-IFN-γ antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Interferon-γ recognizes recombinant rat IFN-γ by various immunochemical techniques including neutralization, immunoblotting, ELISA, and immunohistochemistry. Anti-IFN-γ neutralizes the biological activity of rat IFN-γ and also mouse IFN-γ with the same effectiveness. It will not neutralize the biological activity of human IFN-γ. Based on ELISA and immunoblotting (non-reducing and reducing conditions), this antibody shows almost 100 % cross-reactivity with recombinant mouse IFN-γ and less than 10 % cross-reactivity with recombinant human IFN-γ.

Mature IFN-γ exists as noncovalently linked homodimers. The N-terminal methionyl form of rat IFN-γ contains 135 amino acid residues and has a predicted molecular mass of approximately 15.5 kDa.

Interferon-γ, a type II or immune interferon [1], is produced primarily by T lymphocytes and natural killer cells stimulated by alloantigens, tumors, and mitogens.[2] IFN-γ exerts a variety of biological effects including antiviral activity [3], inhibition of cell or tumor growth [4], and promotion of differentiation of B cells into immunoglobulin-producing cells.[5] IFN-γ, originally characterized based on its antiviral activities, is a potent modulator of immune response and modifies cellular processes.[6] It functions as an activating factor priming macrophages for non-specific tumorcidal activity.[7] IFN-γ activates monocytes to exert enhanced cytotoxicity against tumor cells [8]. It also boosts cytotoxicity of natural killer cells and stimulates T cell cytotoxicity. IFN-γ acts as a signal for the major histocompatibility antigen expression system.[9] The species specificity of IFN-γ resides in the interaction of IFN-γ with its receptor.[10]

Reagent

Supplied as a lyophilized powder from a 0.2 μm filtered solution of PBS 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 0.5 mL of sterile phosphate buffered saline (PBS) to produce a 0.2 mg/ml stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in “frost-free” freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

To measure the ability of this antibody to neutralize the bioactivity of rat IFN-γ on mouse L-929 cells[11], rat IFN-γ is added to various concentrations of the antibody. This mixture is added to confluent cultures of L-929 cells in a 96 well plate. The assay mixture in a total volume of 100 μL, containing antibody at concentrations from 0.001 to 10 μg/mL and recombinant rat IFN-γ at 2.5 ng/mL, is incubated at 37 °C for 20-24 hours in a humidified CO_2 incubator.

At the end of this incubation period, medium is aspirated from all wells and a titrated amount of Encephalomyocarditis virus (EMCV) in prewarmed culture medium is added to each well. After an additional 20-24 hour incubation period, the cells are 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 Anti-IFN-γ is 0.08-0.2 μg/mL in the presence of 2.5 ng/mL of recombinant rat IFN-γ, measuring the protection of mouse L-929 cells from the lytic effect of the virus.

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 recombinant rat IFN-γ activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working antibody concentration of 0.1-0.2 μg/mL is recommended. The detection limit for recombinant rat IFN-γ and recombinant mouse IFN-γ is ~5 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a working antibody concentration of 5-15 μg/mL is recommended to detect rat IFN-γ in treated cultured cells or tissue sections.

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

Endotoxin level is < 0.1 EU/mg antibody as determined by the LAL (Limulus amebocyte lysate) method.

References

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