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Product Information

Anti-Interferon-γ Receptor 1 (IFN-γ R1)
produced in goat, affinity isolated antibody

Catalog Number **I5152**

Product Description

Anti-Interferon-γ Receptor 1 (IFN-γ R1), also called IFN-γ Rα and CDw119, is produced in goat using as immunogen a purified recombinant human IFN-γ R1 extracellular domain, expressed in mouse NSO cells.[1] Affinity isolated antibody is obtained from goat anti-IFN-γ R1 antiserum by immunospecific purification that removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-IFN-γ R1 recognizes recombinant human IFN-γ R1 by various immunochemical techniques, including neutralization and immunoblotting. Based on immunoblotting, this antibody shows no cross-reactivity with recombinant human IFN-γ R2. Human IFN-γ R1, a 227 amino acid residue protein, has a calculated molecular mass of ~25 kDa. As a result of glycosylation, the recombinant IFN-γ R1 migrates as a 40-50 kDa protein in SDS-PAGE. Human and mouse IFN-γ R1 share approximately 52 % amino acid sequence identity and each interacts with IFN-γ in a strictly species-specific manner.[2,3]

The IFN-γ receptor complex is composed of two type I membrane proteins, IFN-γ R1 (IFN-γ Rα) and IFN-γ R2 (IFN-γ Rβ).[4] Both receptor proteins are members of the type II cytokine receptor family and share 52% overall sequence identity. The IFN-γ receptor is constitutively expressed in most cell types. Soluble IFN-γ has been detected in biological fluids. IFN-γ R1 is required for ligand binding and signaling. It binds to IFN-γ with high affinity and is a potent IFN-γ antagonist. In signal transduction, IFN-γ induces tyrosine phosphorylation of IFN-γ R1, leading to the formation of a docking site on the activated receptor for Stat1, which specifically activates IFNγ-induced gene transcription.[2,5,6]

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.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of sterile phosphate buffered saline (PBS) to produce a 0.1 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 at least one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

Product Profile

To measure the ability of this antibody to block the cell surface recombinant human IFN-γ R1 mediated bioactivity on HeLa cells,[7] various concentrations of the antibody are added to confluent cultures of HeLa cells in a 96 well plate. The assay mixture, in a total volume of 150 μL, containing antibody at concentrations from 0.01–50 μg/mL, is incubated for 1 hour at 37 °C. Recombinant human IFN-γ is added at 2 ng/mL and the mixture is incubated at 37 °C for 20 to 24 hours in a humidified CO_2 incubator. At the end of this incubation period, the medium is aspirated from the wells and a titrated amount of Encephalomyocarditis virus (EMCV) in prewarmed culture medium is added to each test well. After an additional 20 to 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-human IFN-γ receptor 1 is 0.5-2.0 μg/mL in the presence of 2 ng/mL of recombinant human IFN-γ, using a confluent culture of HeLa cells.

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 human IFN-γ R1 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working concentration of 0.1 μg/mL antibody is recommended. The detection limit for recombinant human IFN-γ R1 is ~5 ng/lane under non-reducing and reducing conditions.

Immunocytochemistry: a working concentration of 5-15 μg/mL antibody is recommended.

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

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

References

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