Human IFN-γ Antibody

Monoclonal Mouse IgG_{2A} Clone # K3.53

Catalog Number: MAB2852

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human IFN-γ in direct ELISAs and Western blots. In Western blots, no cross-reactivity with recombinant mouse IFN-γ, recombinant rat IFN-γ, recombinant porcine IFN-γ, recombinant feline IFN-γ, or recombinant cotton rat IFN-γ is observed.
Source	Monoclonal Mouse IgG_{2A} Clone # K3.53
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human IFN-γ
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS and NaCl with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

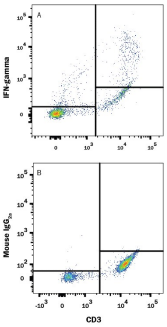
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	Recombinant Human IFN-γ (Catalog # 285-IF)
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Human IFN-γ Sandwich Immunoassay		**Reagent**
ELISA Capture	2-8 µg/mL	Human IFN-γ Antibody (Catalog # MAB2852)
ELISA Detection	0.1-0.4 µg/mL	Human IFN-γ Biotinylated Antibody (Catalog # BAF285)
Standard		Recombinant Human IFN-γ (Catalog # 285-IF)
Neutralization	Measured by its ability to neutralize IFN-γ inhibition of EMCV-induced cytopathy in the HeLa human cervical epithelial carcinoma cell line. Meager, A. (1987) in Lymphokines and Interferons, a Practical Approach. Clemens, M.J. *et al.* (eds): IRL Press. 129. The Neutralization Dose (ND_{50}) is typically ≤2 µg/mL in the presence of 1 ng/mL Recombinant Human IFN-γ.	

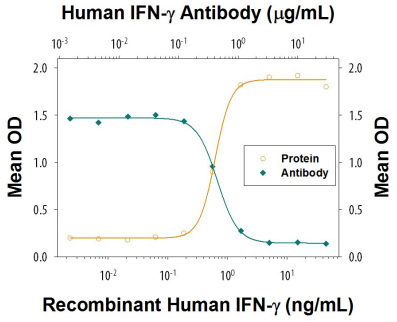
DATA

Intracellular Staining by Flow Cytometry



Detection of IFN- gamma in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) treated with 50 ng/mL PMA, 1 ug/mL Ionomycin, and 3 uM Monensin overnight were stained with either (A) Mouse Anti-Human IFN- gamma Monoclonal Antibody (Catalog # MAB2852) or (B) Mouse IgG2A Isotype Control (Catalog # MAB003) followed by anti-Mouse IgG PE-conjugated secondary antibody (Catalog # F0102B) and Mouse Anti-Human CD3 epsilon APC-conjugated Monoclonal Antibody (Catalog # FAB100A). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012).View our protocol for Staining Intracellular Molecules.

Neutralization



IFN-γ Inhibition of EMCV-induced Cytopathy and Neutralization by Human IFN-γ Antibody. Recombinant Human IFN-γ (Catalog # 285-IF) reduces the Encephalomyocarditis Virus (EMCV)-induced cytopathy in the HeLa human cervical epithelial carcinoma cell line in a dose-dependent manner (orange line), as measured by crystal violet staining. Inhibition of EMCV activity elicited by Recombinant Human IFN-γ (1 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human IFN-γ Monoclonal Antibody (Catalog # MAB2852). The ND_{50} is typically ≤2 µg/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Interferon-gamma (IFN-γ), also known as type II or immune interferon, exerts a wide range of immunoregulatory activities and is considered to be the prototype proinflammatory cytokine (1, 2). Mature human IFN-γ exists as a non-covalently linked homodimer of 20-25 kDa variably glycosylated subunits (3). It shares 90% amino acid (aa) sequence identity with rhesus IFN-γ, 59-64% with bovine, canine, equine, feline, and porcine IFN-γ, and 37-43% with cotton rat, mouse, and rat IFN-γ. IFN-γ dimers bind to IFN-γ RI (alpha subunits) which then interact with IFN-γ RII (beta subunits) to form the functional receptor complex of two α and two β subunits. Inclusion of IFN-γ RII increases the binding affinity for ligand and the efficiency of signal transduction (4, 5). IFN-γ is produced by a variety of immune cells under inflammatory conditions, notably by T cells and NK cells (6). It plays a key role in host defense by promoting the development and activation of Th1 cells, chemoattraction and activation of monocytes and macrophages, upregulation of antigen presentation molecules, and immunoglobulin class switching in B cells. It also exhibits antiviral, antiproliferative, and apoptotic effects (6, 7). In addition, IFN-γ functions as an anti-inflammatory mediator by promoting the development of regulatory T cells and inhibiting Th17 cell differentiation (8, 9). The pleiotropic effects of IFN-γ contribute to the development of multiple aspects of atherosclerosis (7).

References:

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