**DESCRIPTION**

Species Reactivity: Human

Specificity: Detects human IFN-γ.

Source: Monoclonal Mouse IgG2A Clone # 25718

Purification: Protein A or G purified from hybridoma culture supernatant

Immunogen: E. coli-derived recombinant human IFN-γ Gln24-Gln166 Accession #: AAP20098.1

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 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**

General Protocols are available in the Technical Information section on our website.

**Recommended Concentration**

**Sample**

<table>
<thead>
<tr>
<th>Immunocytochemistry</th>
<th>8-25 µg/mL</th>
<th>See Below</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular Staining by Flow Cytometry</td>
<td>0.25 µg/10^6 cells</td>
<td>See Below</td>
</tr>
</tbody>
</table>

**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 (ND50) is typically 0.02-0.06 µg/mL in the presence of 1 ng/mL Recombinant Human IFN-γ.

---

**DATA**

**Immunocytochemistry**

IFN-γ in Human PBMCs. IFN-γ was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) stimulated with PMA and ionomycin using 10 µg/mL Human IFN-γ Monoclonal Antibody (Catalog # MAB285) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

**Intracellular Staining by Flow Cytometry**

Detection of IFN-γ 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 # MAB285) 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 Human IFN-γ Monoclonal Antibody (Catalog # MAB285). The ND50 is typically 0.02-0.06 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.

**BACKGROUND**
Interferon-gamma (IFN-gamma, IFNG), also known as type II or Immune Interferon, exerts a wide range of immunoregulatory activities and is considered to be the prototype proinflammatory cytokine. Mature human IFN-gamma exists as a non-covalently linked homodimer of 20-25 kDa molecular weight variably glycosylated subunits. Glycosylation of IFN-gamma at sites Asn25 and Asn97 is critical for protease resistance. It shares 90% amino acid (aa) sequence identity with rhesus IFN-gamma, 59-64% with bovine, canine, equine, feline, and porcine IFN-gamma, and 37-43% with cotton rat, mouse, and rat IFN-gamma. IFN-gamma dimers bind to IFN-gamma RI (alpha subunits) which then interact with IFN-gamma RII (beta subunits) to form the functional receptor complex of two alpha and two beta subunits. Inclusion of IFN-gamma RII increases the binding affinity for ligand and the efficiency of signal transduction. IFN-gamma is produced by a variety of immune cells under inflammatory conditions, notably by T cells and NK cells. It plays a key function in host defense by promoting the development and activation of Th1 cells, chemoattraction and activation of monocytes and macrophages, up-regulation of antigen presentation molecules, and immunoglobulin class switching in B cells. It also exhibits antiviral, antiproliferative, and apoptotic effects. In addition, IFN-gamma functions as an anti-inflammatory mediator by promoting the development of regulatory T cells and inhibiting Th17 cell differentiation. The pleiotropic effects of IFN-gamma contribute to the development of multiple aspects of atherosclerosis.