

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse IFN- γ in Western blots. In Western blots, this antibody does not cross-react with recombinant human (rh) IFN- γ , rIFN- γ , rplIFN- γ , rrmIFN- γ , rfeIFN- γ , or rcalIFN- γ .
Source	Monoclonal Rat IgG _{2A} Clone # 37895
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant mouse IFN- γ
Endotoxin Level	<0.10 EU per 1 μ g of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 μ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μ m 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 μ g/mL	Recombinant Mouse IFN- γ (Catalog # 485-MI)
Intracellular Staining by Flow Cytometry	0.25 μ g/10 ⁶ cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize IFN- γ inhibition of EMCV-induced cytopathy in the L-929 mouse fibroblast cell line. Vogel, S. and M. Hogan (1995) in <i>Current Protocols in Immunology</i> . Ciocio, R. (ed); John Wiley & Sons, Inc. p. 6. 9. 1. The Neutralization Dose (ND ₅₀) is typically 0.075-0.3 μ g/mL in the presence of 2.5 ng/mL Recombinant Mouse IFN- γ .	

DATA

Intracellular Staining by Flow Cytometry

Detection of IFN- γ in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes either (A) stimulated to induce Th1 cells or (B) unstimulated were stained with Rat Anti-Mouse IFN- γ Monoclonal Antibody (Catalog # MAB485) followed by Phycoerythrin-conjugated Anti-Rat IgG Secondary Antibody (Catalog # F0105B) and Rat Anti-Mouse CD4 APC-conjugated Monoclonal Antibody (Catalog # FAB554A). Quadrant markers were set based on control antibody staining (Catalog # MAB006). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for [Staining Intracellular Molecules](#).

Neutralization

IFN- γ Inhibition of EMCV-induced Cytopathy and Neutralization by Mouse IFN- γ Antibody. Recombinant Mouse IFN- γ (Catalog # 485-MI) reduces the Encephalomyocarditis Virus (EMCV)-induced cytopathy in the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line), as measured by crystal violet staining. Inhibition of EMCV activity elicited by Recombinant Mouse IFN- γ (2.5 ng/mL) is neutralized (green line) by increasing concentrations of Rat Anti-Mouse IFN- γ Monoclonal Antibody (Catalog # MAB485). The ND₅₀ is typically 0.075-0.3 μ g/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. <ul style="list-style-type: none"> • 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- γ), also known as type II or immune interferon, exerts a wide range of immunoregulatory activities and is considered to be the prototype pro-inflammatory cytokine (1, 2). Mature mouse IFN- γ exists as a noncovalently linked homodimer of 20-25 kDa variably glycosylated subunits (3). It shares 86% amino acid sequence identity with rat IFN- γ and 38-44% with bovine, canine, cotton rat, equine, feline, human, porcine, and rhesus macaque 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 anti-viral, anti-proliferative, 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:

1. Billiau, A. and P. Matthys (2009) Cytokine Growth Factor Rev. **20**:97.
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3. Gray, P.W. and D.V. Goeddel (1983) Proc. Natl. Acad. Sci. USA **80**:5842.
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