Technical Data Sheet

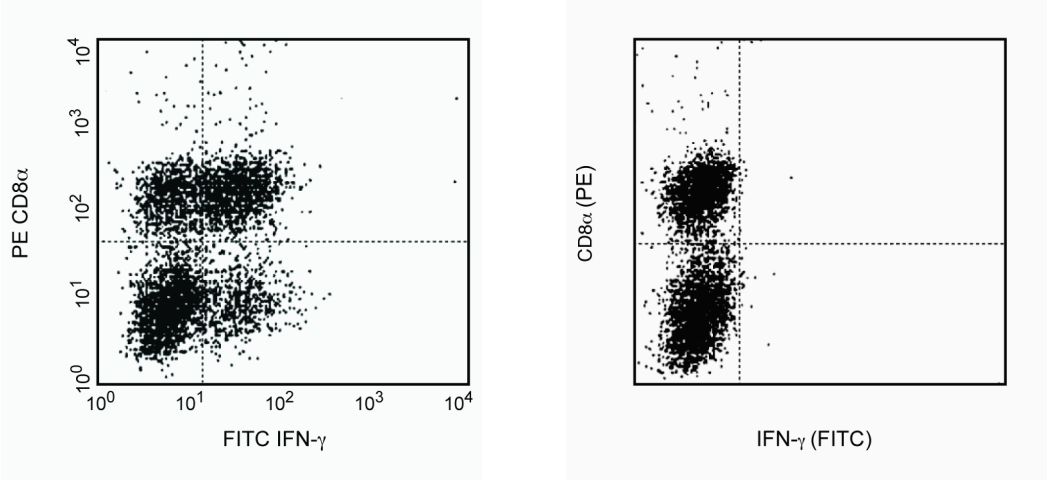
FITC Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**562019**
Alternate Name:	IFN-γ; IFN-g; IFN-gamma; Interferon gamma; Type II Interferon
Size:	25 µg
Concentration:	0.5 mg/ml
Clone:	XMG1.2
Immunogen:	Mouse IFN-γ Recombinant Protein
Isotype:	Rat IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The XMG1.2 monoclonal antibody specifically binds to mouse interferon-γ (IFN-γ) protein. IFN-γ is a pleiotropic cytokine, of approximately 15-17 kDa, involved in the regulation of inflammatory and immune responses. It plays an important role in activation, growth, and differentiation of T and B lymphocytes, macrophages, NK cells and other non-hematopoietic cell types. IFN-γ production is associated with the Th1 cell differentiation. The purified form of this antibody has been reported to be a neutralizing antibody.



Expression of IFN-γ by stimulated CD8+ and CD8- BALB/c spleen cells. *BALB/c spleen cells were cultured for 72h in medium containing Staphylococcus aureus entertoxin B (SEB, 2 µg/ml; Sigma Cat. No. S-4881), recombinant mouse IL-2 (10 U/ml, BD Cat. No. 550069) and recombinant mouse IL-4 (2 ng/ml, BD Cat. No. 550067). The cells were harvested and restimulated for 5 h with immobilized anti-CD3 (145-2C11, BD Cat. No. 553057 at 10 µg/ml) and anti-CD28 (clone 37.51, BD Cat. No. 553294 at 2 µg/ml) antibodies in the presence of BD GolgiStop™ (2 µM final concentration, Cat. No. 554724). The splenocytes were stained with 0.25 µg of PE rat anti-mouse CD8 (PE-53-6.7, Cat. No. 553033), fixed permeabilized, and subseqently stained with 0.1 µg of FITC rat anti-mouse IFN-γ (FITC-XMG1.2, Cat. No. 554411 - left panel). To demonstrate specificity of staining, the binding by FITC-XMG1.2 was blocked by preincubation of the fixed/permeabilized cells with excess unlabeled XMG1.2 mAb (5 µg; Cat. No. 554409, right panel) prior to staining with the FITC-XMG1.2. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabelled antibody blocking specificity control.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The FITC-conjugated XMG1.2 antibody can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate IFN-γ producing cells within mixed cell populations. For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤0.5 µg mAb/million cells).

A useful control for deomonstrating specificity of staining is either of the following: (1) pre-block the conjugated XMG1.2 antibody with a molar excess of ligand (e.g., recombinant mouse IFN-γ; Cat. No. 554587) prior to staining, or (2) pre-block the fixed/permeabilized cells with un-conjugated XMG1.2 antibody (Cat. No. 554409) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcelfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse cells is FITC-R3-34 (Cat. No. 554684); use at comparable concentrations to antibody of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
550069	Recombinant Mouse IL-2	20 µg	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554684	FITC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
550067	Recombinant Mouse IL-4	10 µg	(none)
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. An isotype control should be used at the same concentration as the antibody of interest.

References

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific)

Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med.* 1987; 166(5):1229-1244. (Clone-specific)

Ferrick DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, Lepper H. Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. *Nature.* 1995; 373(6511):255-257. (Clone-specific: Flow cytometry)

Hsieh B, Schrenzel MD, Mulvania T, Lepper HD, DiMolfetto-Landon L, Ferrick DA. In vivo cytokine production in murine listeriosis. Evidence for immunoregulation by gamma delta+ T cells. *J Immunol.* 1996; 156(1):232-237. (Clone-specific: Flow cytometry)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology: Immunofluorescence)

Sander B, Hoiden I, Andersson U, Moller E, Abrams JS. Similar frequencies and kinetics of cytokine producing cells in murine peripheral blood and spleen. Cytokine detection by immunoassay and intracellular immunostaining. *J Immunol Methods.* 1993; 166(2):201-214. (Clone-specific)

Vikingsson A, Pederson K, Muller D. Enumeration of IFN-gamma producing lymphocytes by flow cytometry and correlation with quantitative measurement of IFN-gamma. *J Immunol Methods.* 1994; 173(2):219-228. (Clone-specific: Flow cytometry)