Technical Data Sheet

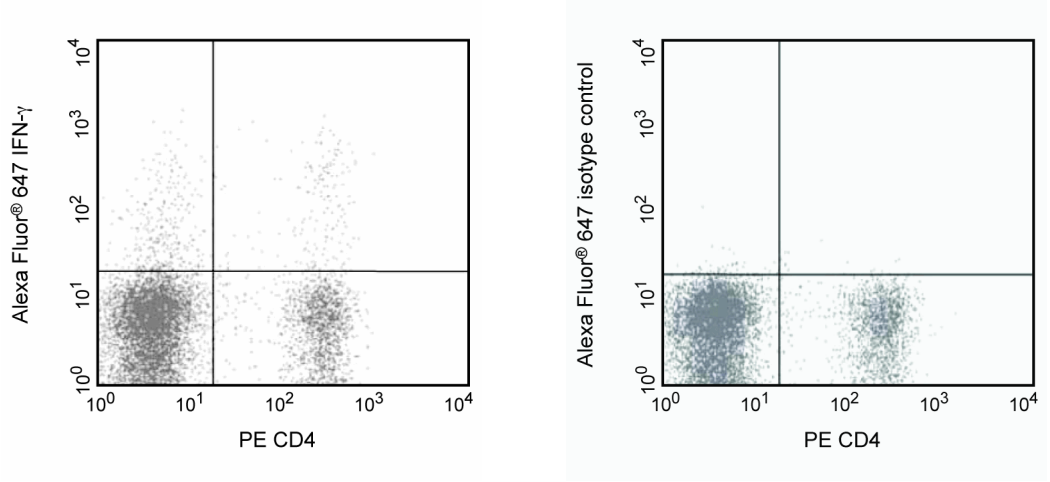
Alexa Fluor® 647 Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**557735**
Alternate Name:	IFN-γ; IFN-g; IFN-gamma; Interferon gamma; Type II Interferon
Size:	0.1 mg
Concentration:	0.2 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.



Analysis for IFN-γ in stimulated CD4+ and CD4 BALB/c spleen cells. *Splenocytes from BALB/C mice were stimulated for 4 hrs with PMA (5 ng/ml, Sigma Cat. No. P-8139) and Ionomycin (500 ng, Sigma Cat. No. I-0634) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with PE Rat Anti-Mouse CD4 (Cat. No. 553048) and either the Alexa Fluor® 647 Rat Anti-Mouse IFN-γ antibody (left panel) or with a Alexa Fluor® 647 Rat IgG1 isotype control (Cat. No. 557731), (right panel). To demonstrate specificity of the staining, the binding of the Alexa Fluor® 647 Rat Anti-Mouse IFN-γ antibody (XMG1.2) was blocked by preincubation of the fixed/permeabilized cells with an excess of unlabelled XMG1.2 antibody (5 µg, Cat. No. 554409, data not shown) prior to stainining. The quadarant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.*

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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
557731	Alexa Fluor® 647 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
553048	PE Rat Anti-Mouse CD4	0.1 mg	RM4-5

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554409	Purified Rat Anti-Mouse IFN-γ	0.1 mg	XMG1.2
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. 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.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. An isotype control should be used at the same concentration as the antibody of interest.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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. (Biology)

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. (Biology)

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: Flow cytometry)

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. (Biology)

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