Technical Data Sheet

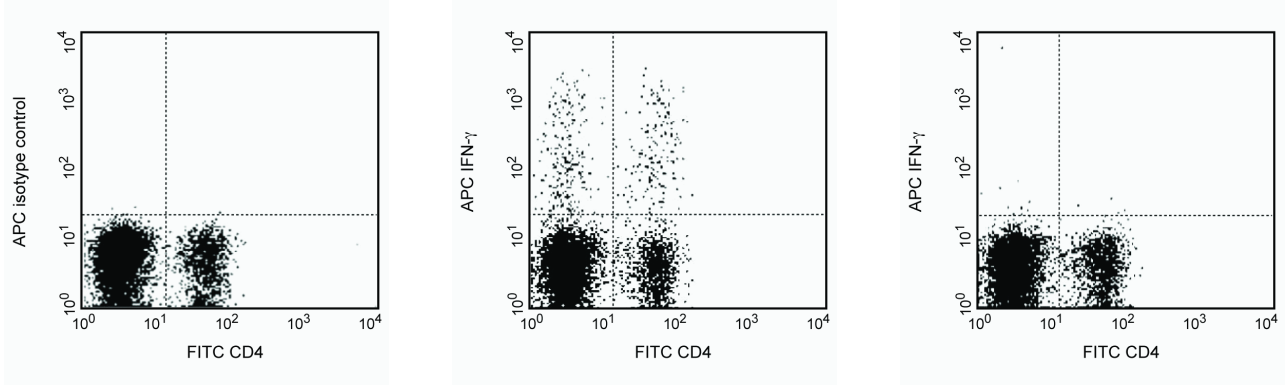
APC Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**554413**
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
Target MW:	15-17 kDa
Storage Buffer:	Aqueous buffered solution containing ≤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 CD4+ and CD4-C3H spleen cells. *Splenocytes from C3H mice were stimulated in culture for 4 hours using PMA (5 ng/ml final concentration; Sigma Cat. #P-8139) and Ionomycin (500 ng/ml final concentration; Sigma Cat. #I0634) in the presence of GolgiPlug™ Protein Transport Inhibitor (1 µl/ml, Cat. No. 555029). The splenocytes were harvested and stained with 0.06 µg of FITC Rat Anti-Mouse CD4 (FITC-Rm4-5, Cat. No. 553047), fixed, permeabilized and subsequently stained with 0.12 µg of APC Rat IgG1 isotype control antibody (APC-R3-34, Cat. No. 554686, left panel) or with APC Rat Anti-Mouse IFN-γ (APC-XMG1.2, Cat. No. 554413, middle panel) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining, the binding by the APC-XMG1.2 antibody was blocked by preincubation of the fixed, premeabilized cells with unlabeled XMG1.2 antibody (5.0 µg; Cat. No. 554409, right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the unlabeled antibody blocking specificity control.*

Preparation and Storage

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

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

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Immunofluorescent Staining for Flow Cytometric Analysis: The APC-XMG1.2 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. For optimal immunofluorescent staining for flow cytometric analysis, the anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse cells is APC-R3-34 (Cat. No. 554686); use at comparable concentrations to antibody of interest. A useful control for demonstrating specificity of staining

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is either of the following: (1) pre-block the APC-conjugated XMG1.2 antibody with ligand (e.g., recombinant mIFN-γ, Cat. No. 554587) prior to staining, or (2) pre-block the fixed/permeabilized cells with unlabeled XMG1.2 antibody (Cat. No. 554409) prior to staining.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554686	APC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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.

References

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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)

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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. (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)