Technical Data Sheet

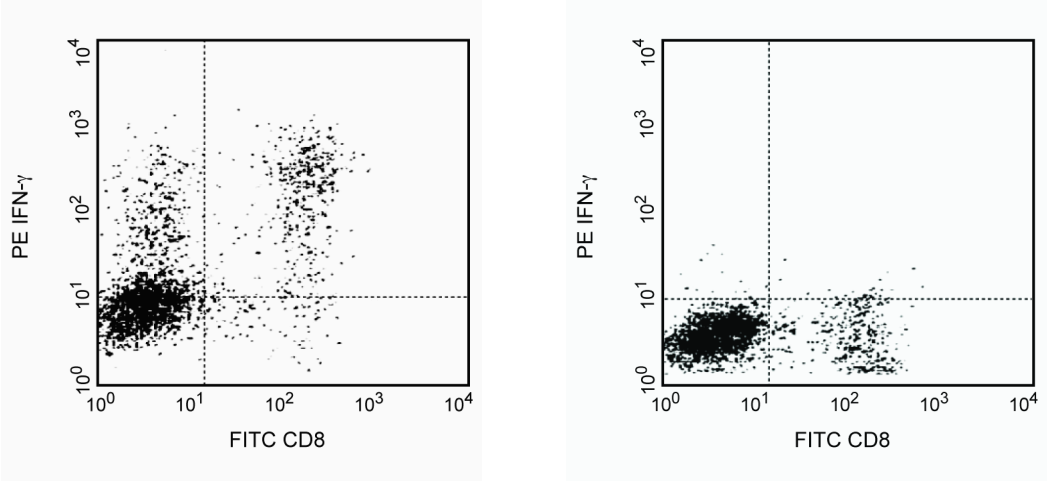
PE Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**562020**
Size:	25 µg
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 ≤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. *Splenocytes from 6 month old BALB/c mice were cultured for 3 days in the presence of SEB (2 µg/ml; Sigma, Cat. No. S-4881), then restimulated for 5 hour with hamster anti-mouse CD3 (2 µg/ml, 145-2C11, Cat. No. 553057) and hamster anti-mouse CD28 (2 µg/ml, 37.51, Cat. No, 553294) antibodies in the presence of 2 µM GolgiStop™ (aka, monensin; Cat. No. 554724). The splenocytes were harvested, stained with 0.06 µg of FITC rat anti-mouse CD8 (FITC 53-6.7, Cat. No. 553030), fixed, permeabilized, and subsequently stained with 0.06 µg of PE rat anti-mouse IFN-γ (PE-XMG1.2, Cat. No. 554412, left panel) by using Pharmingen's staining protocol. To demonstrate specificity of staining, the binding by the PE-XMG1.2 antibody was blocked by preincubation of the fixed/permeabilized cells with unlabeled XMG1.2 antibody (5.0 µg; see right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabeled antibody blocking specificity 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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The XMG1.2 antibody is useful for the immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine PE antibody should be titrated (≤ 0.5 µg mAb/million cells). A useful control for demonstrating specificity of staining is either of the following: (1) pre-block the fluorochrome-conjugated XMG1.2 antibody prior to staining, with unlabeled XMG1.2 antibody (Cat. No. 554409), or (2) pre-block with a molar excess of ligand, (e.g. rec. ms. IFN-g; Cat. No. 554587) prior to staining.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Suggested Companion Products

Catalog Number	Name	Size	Clone
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
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. 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.
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)

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: Blocking, Neutralization)

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)