

Technical Data Sheet

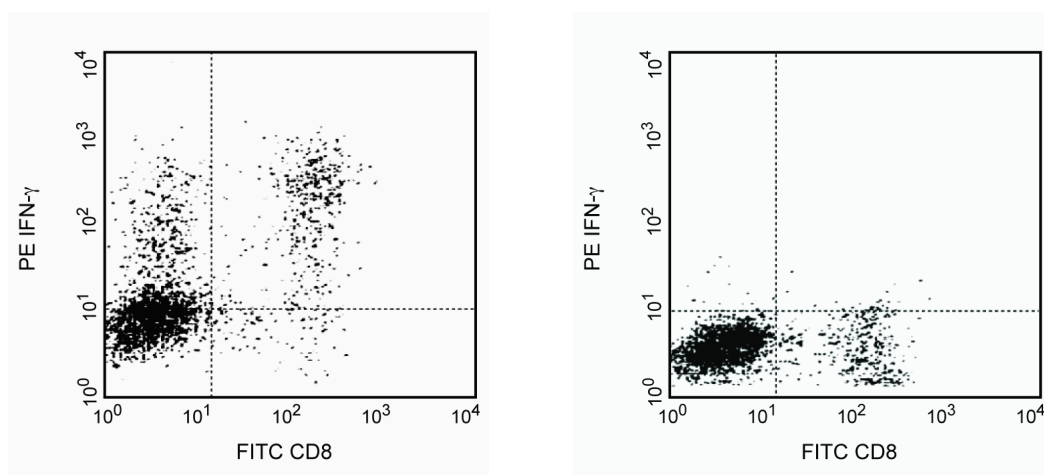
PE Rat Anti-Mouse IFN- γ

Product Information

Material Number:	554412
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 $\leq 0.09\%$ 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 μ g/ml; Sigma, Cat. No. S-4881), then restimulated for 5 hour with hamster anti-mouse CD3 (2 μ g/ml, 145-2C11, Cat. No. 553057) and hamster anti-mouse CD28 (2 μ g/ml, 37.51, Cat. No. 553294) antibodies in the presence of 2 μ M GolgiStop™ (aka, monensin; Cat. No. 554724). The splenocytes were harvested, stained with 0.06 μ g of FITC rat anti-mouse CD8 (FITC 53-6.7, Cat. No. 553030), fixed, permeabilized, and subsequently stained with 0.06 μ g 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 μ g; 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

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.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The XMG1.2 antibody is useful for the immunofluorescent staining and flow

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cytometric analysis to identify and enumerate IFN- γ producing cells within mixed cell populations. The PE-conjugated XMG1.2 antibody is especially suitable for these studies. For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated ($\leq 0.5 \mu\text{g mAb/million cells}$). 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. recombinant mouse IFN-g; Cat. No. 554587) prior to staining.

A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells is PE-R3-34 immunoglobulin (Cat. No. 554685); use at comparable concentrations to antibody of interest.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
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
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytotfix/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 wwwbdbiosciences.com/pharming/en/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at wwwbdbiosciences.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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- 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)