Technical Data Sheet

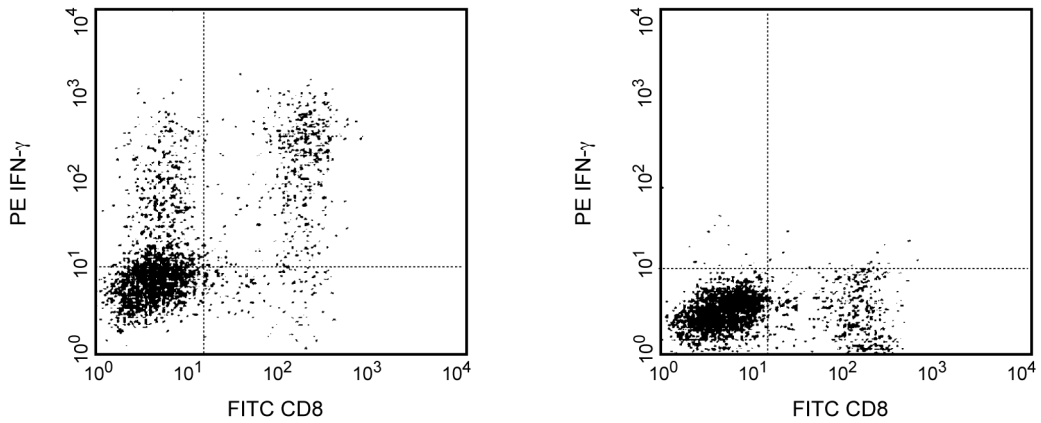
Purified Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**554409**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	XMG1.2
Immunogen:	Mouse IFN-γ
Isotype:	Rat IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The XMG1.2 antibody reacts with mouse interferon-γ (IFN-γ) protein. IFN-γ is a pleiotropic cytokine, of approximately 15-17 kDa, involved in the regulation of the immune response. 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 Th-1 differentiation. This is 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 hr with hamster anti-mouse CD3 (2 µg/ml, 145-2C11, Cat. #553057) and hamster anti-mouse CD28 (2 µg/ml, 37.51, Cat. No. 553294) antibodies in the presence of 2 µg GolgiStop™ (aka, Monensin; Cat. No. 554724). The splenocytes were harvested, stained with 0.06 µg of FITC-conjugated rat anti-mouse CD48 (FITC-53-6.7 Cat. No. 553047), fixed, permeabilized, and subsequently stained with 0.06 µg of PE-conjugated rat anti-mouse IFN-γ antibody (PE-XMG1.2) by using Pharmingen's staining protocol (left panel). 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; 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.
Store undiluted at 4°C.

Application Notes

Application

ELISA	Routinely Tested
Neutralization	Tested During Development
Intracellular block/flow cytometry	Tested During Development
Immunocytochemistry (cytospins)	Tested During Development
Western blot	Reported

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Immunofluorescent Staining and Flow Cytometric Analysis: The purified XMG1.2 antibody can be used as a blocking control to demonstrate specificity of IFN-γ staining by directly conjugated-XMG1.2 (PE: Cat. No. 554412; FITC: Cat. No. 554411; APC: Cat. No. 554413; PE-Cy7: Cat. No. 557649). To perform this control, the fixed/permeabilized cells (~1 million) can be incubated with 1-10 µg of unlabeled XMG1.2 antibody (Cat. No. 554409) for 20 minutes at 4°C, prior to staining with conjugated-XGM1.2 antibody.

Western blot: The purified XMG1.2 antibody (Cat. No. 554409) has been reported to be useful for Western blotting. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

ELISA Detection: The biotinylated XMG1.2 antibody (Cat. No. 554410) is useful as a detection antibody for a sandwich ELISA for measuring mouse IFN-γ protein levels and can be used in combination with purified R4-6A2 (Cat. No. 551216) as the capture antibody and recombinant mouse IFN-γ as the standard. This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. These ELISA reagents are not recommended for assaying serum or plasma samples. For measuring Mouse IFN-γ in serum or plasma our Mouse IFN-γ BD OptEIA™ Set (Cat. No. 551866) or BD OptEIA Kit (Cat. No. 558258) are specially formulated and recommended.

Neutralization/Blocking: The NA/LE™ format of the XMG1.2 antibody (Cat. No. 554408) is useful for neutralization of mouse IFN-γ bioactivity.

Suggested Companion Products

Catalog Number	Name	Size	Clone
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
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	5x10^6 cells	(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. 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

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)

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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: Blocking, 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)