Technical Data Sheet

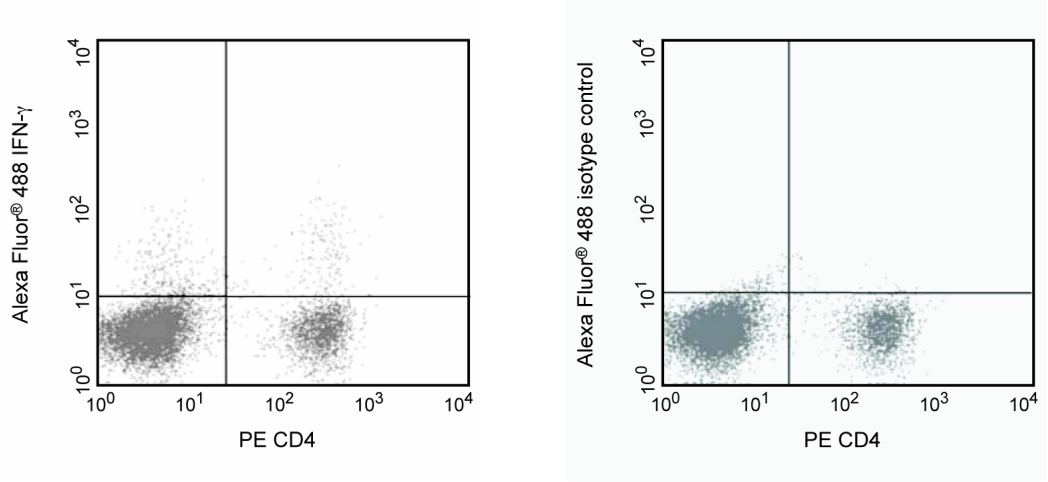
Alexa Fluor® 488 Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**557724**
Size:	0.1 mg
Concentration:	0.2 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 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 (PE-RM4-5, Cat. No. 553048) and either rat anti-mouse IFN-γ (Alexa Fluor® 488-XMG1.2, Cat. No. 557724), (left panel) or immunoglobulin isotype control (Alexa Fluor® 488-R3-34, Cat. No. 557720), (right panel) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining the binding of Alexa Fluor® 488-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

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was 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 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. The Alexa Fluor® 488-conjugated XMG1.2 antibodies (Cat. No. 557724) is especially suitable for these studies. For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated. A useful control for demonstrating specificity of staining is to pre-block the fixed/permabilized cells

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with unlabeled XMG1.2 antibody (Cat. No. 554409) 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 R3-34 immunoglobulin (Cat. No. 557720). Use at comparable concentrations to antibody of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557720	Alexa Fluor® 488 Rat IgG1 κ Isotype Control	0.1 mg	R3-34
554652	MiCK-1 Mouse Cytokine Positive Control Cells	5x10^6 cells	(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. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. 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.
6. 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.
7. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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