Technical Data Sheet

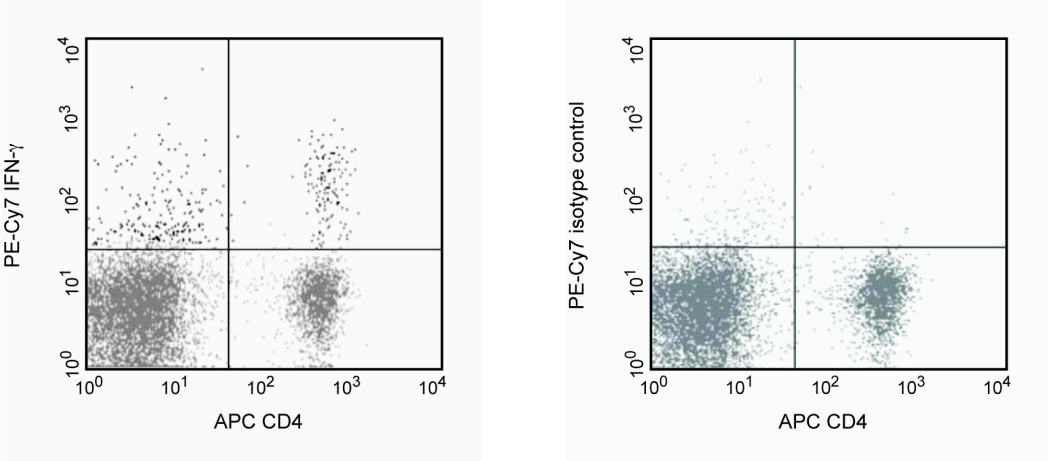
PE-Cy™7 Rat Anti-Mouse IFN-γ

Product Information

Material Number:	**561040**
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 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.



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 µg/ml, Sigma Cat. No. I-0634) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with APC rat anti-mouse CD4 (APC-RM4-5, Cat. No. 553051) and either rat anti-mouse IFN-γ antibody (PE-Cy7-XMG1.2, Cat. No. 557649), (left panel) or immunoglobulin isotype control (PE-Cy7-R3-34, Cat. No. 557645), (right panel) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining the binding of PE-Cy7-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

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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-Cy-7 conjugated 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 antibody should be titrated (≤ 0.125 µg mAb/million cells). A useful control for demonstrating specificity of staining is to pre-block the fixed/permeabilized cells 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 PE-R3-34 immunoglobulin (Cat. No. 557645); use at comparable concentrations to antibody of interest.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557645	PE-Cy™7 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. An isotype control should be used at the same concentration as the antibody of interest.
3. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
4. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
7. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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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)

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