Technical Data Sheet

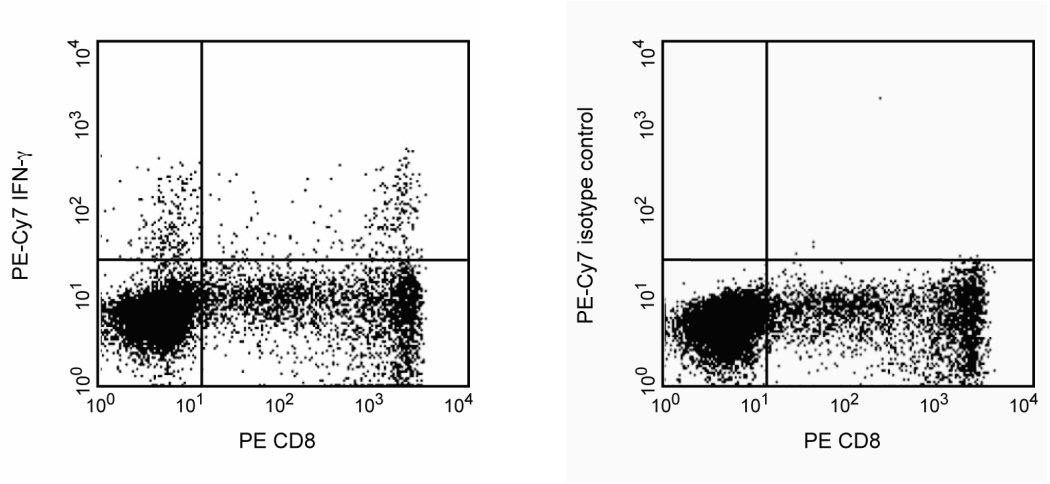
PE-Cy™7 Mouse Anti-Human IFN-γ

Product Information

Material Number:	**557844**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	4S.B3
Immunogen:	Partially purified human IFN-γ from supernatants of human PBMC stimulated with Staphylococcus aureus
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 4S.B3 antibody reacts with human interferon-γ (IFN-γ). The immunogen used to generate this hybridoma was partially purified human IFN-γ obtained from supernatants of human PBMC stimulated with *Staphylococcus aureus*.



Expression of IFN-γ by stimulated human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMCs) were stimulated with PMA and Ionomycin. Stimulated cells were stained with PE Mouse anti-Human CD8 (Clone RPA-T8, Cat. No. 555367) and either PE-Cy™7 Mouse anti-Human IFN-γ (Clone 4S.B3, Cat. No. 557844), (left panel) or PE-Cy™7 mouse IgG1 κ isotype control (Clone MOPC-21, Cat. No. 557646), (right panel) by using the BD Pharmingen™ staining protocol. To demonstrate specificity, PE-Cy™7 Mouse anti-Human IFN-γ binding was blocked when fixed/permeabilized cells were incubated with an excess of purified Mouse anti-Human IFN-γ (5 ug, Clone 4S.B3, Cat. No 554549) prior to staining. Data not shown. Dot plots were derived from gated events with the forward, and side light scatter characteristics of lymphocytes. 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 4S.B3 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. For specific methodology, please visit the protocols section or the intracellular staining chapter in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

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A useful control for demonstrating specificity of staining is the following: pre-block the fixed/permeabilized cells with unlabeled 4S.B3 antibody (Cat. No. 554549) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is PE-Cy™7 Mouse IgG1 κ Isotype Control (Clone MOPC-21, Cat. No. 557646).

Suggested Companion Products

Catalog Number	Name	Size	Clone
557646	PE-Cy™7 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
555367	PE Mouse Anti-Human CD8	100 tests	RPA-T8
554549	Purified Mouse Anti-Human IFN-γ	10 µg	4S.B3

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. 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.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. 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.
8. 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.
9. 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).
10. 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.

References

Meager A. Characterization of interferons and immunoassays. In: Clemens MJ, Morris AG, Gearing AJH, ed. *Lymphockies and Interferons. A Practical Approach.* Oxford: IRL Press Ltd; 1987:105-127. (Biology)

Meager A, Parti S, Barwick S, Spragg J, O'Hagan K. Detection of hybridomas secreting monoclonal antibodies to human gamma interferon using a rapid screening technique and specificity of certain monoclonal antibodies to gamma interferon. *J Interferon Res.* 1984; 4(4):619-625. (Biology)

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)