Technical Data Sheet

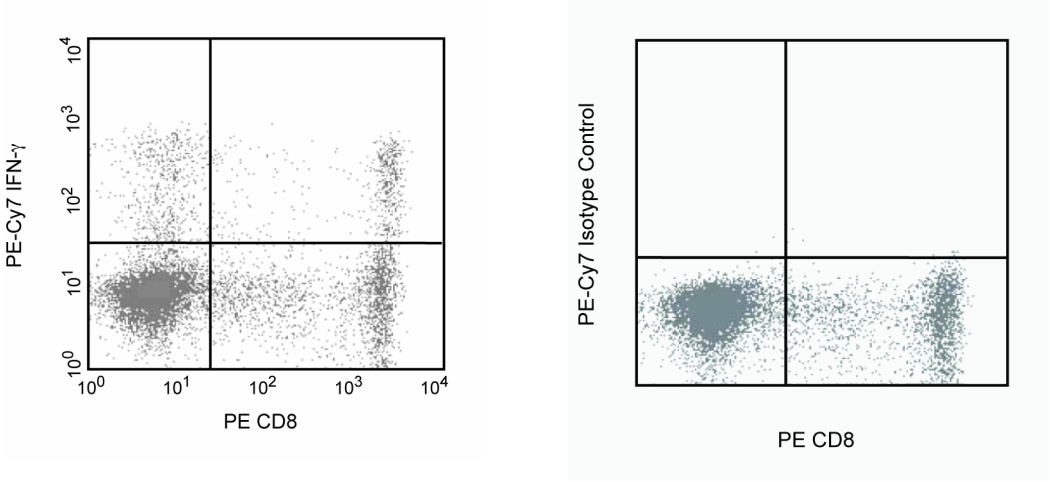
PE-Cy™7 Mouse Anti-Human IFN-γ

Product Information

Material Number:	**560924**
Size:	25 tests
Vol. per Test:	5 µl
Clone:	B27
Immunogen:	Human IFN-γ Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The B27 monoclonal antibody specifically binds to human interferon-γ (IFN-γ). This is a neutralizing antibody. The use of B27 antibody for epitope mapping of human IFN-γ has been described. The B27 antibody has been reported not to bind to denatured IFN-γ.



Expression of IFN-γ by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 4 hrs with PMA (5 ng/ml, Sigma) and Ionomycin (500 ng, Sigma) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with mouse anti-human CD8 (PE RPA-T8, Cat. No. 555367) and either mouse anti-human IFN-γ antibody (PE-Cy7 B27, Cat. No. 557643, left panel) or immunoglobulin isotype control (PE-Cy7 MOPC-21, Cat. No. 557646, right panel) as a specificity control. To demonstrate additional specificity of staining the binding of PE-Cy7 B27 was blocked by preincubation of the fixed/permeabilized cells with an excess of unlabelled B27 antibody (5 µg, Cat. No. 554699, 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 with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 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

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-Cy7-conjugated B27 antibody (Cat. No. 557643) is useful for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IFN-γ producing cells within mixed cell populations. A useful control for demonstrating specificity of staining is the following: pre-block the paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled B27 antibody (Cat. No. 554699) prior to staining. The intracellular cytokine staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

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Neutralization: The NA/LE B27 antibody (Cat. No. 554698) is useful for neutralization of human IFN-γ bioactivity.

IP/WB: The B27 antibody has been reported to be useful for immunoprecipitation studies. The B27 antibody has been reported not to bind to denatured IFN-γ . Please note that these applications are not routinely tested at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
557646	PE-Cy™7 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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. 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.
7. 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.
8. 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.
9. 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.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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. (Biology)

Favre C, Wijdenes J, Cabrillat H, Djossou O, Banchereau J, de Vries JE. Epitope mapping of recombinant human gamma interferon using monoclonal antibodies. *Mol Immunol.* 1989; 26(1):17-25. (Clone-specific: Immunoprecipitation, Neutralization)

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)