Technical Data Sheet

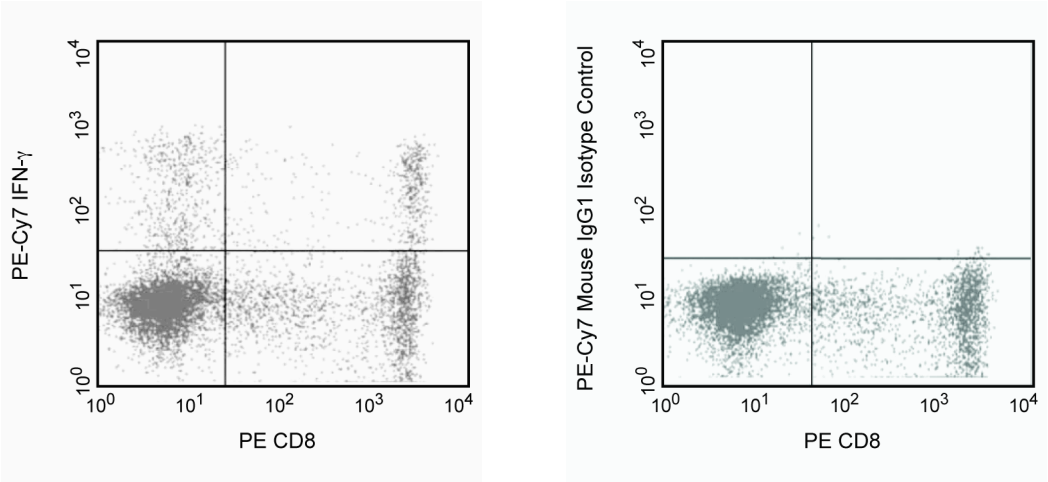
PE-Cy™7 Mouse IgG1 κ Isotype Control

Product Information

Material Number:	**557646**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	MOPC-21
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The MOPC-21 immunoglobulin is a mouse myeloma protein. The MOPC-21 immunoglobulin was selected as an isotype control following screening for low background on a variety of mouse and human tissues.



Ig isotype control for the expression of IFN-γ by stimulated human peripheral blood mononuclear cells (PBMC).
Human PBMC were stimulated for 4 hours 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 PE mouse anti-human CD8 (PE-RPA-T8, Cat. No. 555367) and either mouse anti-human IFN-γ (PE-Cy7-B27, Cat. No. 557643), (left panel) or PE-Cy7-MOPC-21 immunoglobulin (Cat. No. 557646), (right panel) as a specificity control by using Pharmingen's staining protocol. To demonstrate additional specificity of staining the binding of PE-Cy7-B27 was blocked and by preincubation of the fixed/permeabilized cells with an excess of unlabelled B27 antibody (5 µg, Cat. No. 554699) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype control.

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
Flow cytometry	Routinely Tested
Isotype control	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-Cy7-MOPC-21 immunoglobulins (Cat. No. 557646) is suitable mouse IgG1κ isotype controls for assessing the level of background staining on paraformaldehyde fixed/saponin-permeabilized (such as BD Cytofix/Cytoperm™) mouse or human cells for flow cytometric analysis. For specific methodology, visit the protocols section of our website, or refer to the Immune Function Handbook, which is posted on our web site at www.bdbiosciences.com.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)

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. This antibody has been optimized and preassayed with its matched isotype control to be used at the recommended volume of 5 ul/test. Titration of the reagents or substituting with other (non-matched) isotype control is NOT recommended.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. 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).
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. 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.
8. 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.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
10. 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

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)