

Technical Data Sheet

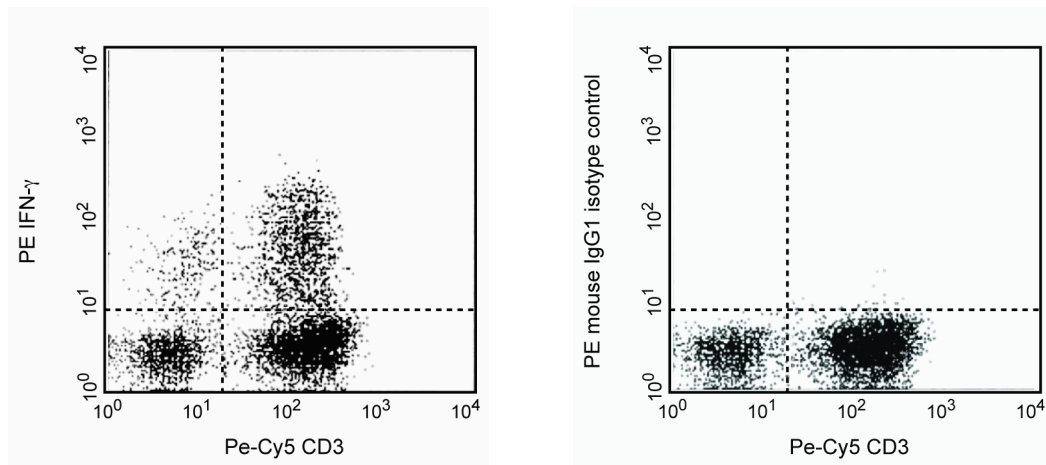
PE Mouse IgG1, κ Isotype Control

Product Information

Material Number:	554680
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	MOPC-21
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing ≤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.



Expression of IFN-γ by stimulated human peripheral blood mononuclear cells (PBMC). Human PBMC were stimulated for 6 hours with PMA (50 ng/ml final concentration; Sigma, Cat. No. P-8139) and calcium ionophore A23187 (Sigma, Cat. No. C-9275), in the presence of GolgiStop™ (2 μM final concentration; Cat. No. 554724). The PBMC were stained with PE-Cy5 anti-CD3 (PE-Cy5-UCHT1, Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.25 μg of PE-mouse anti-human IFN-γ antibody (PE-4S.B3, Cat. No. 554552, left panel) or with 0.25 μg PE-MOPC-21 immunoglobulin (Cat. No. 554680, right panel) using the BD Pharmingen™ staining protocol. To demonstrate specificity of staining, the binding of PE-4S.B3 antibody was blocked by preincubation of fixed/permeabilized cells with excess unlabelled 4S.B3 antibody (5 μg; Cat. No. 554549). The quadrant markers for the bivariate dot plot were set based on autofluorescence controls and verified using the unlabelled 4S.B3 antibody blocking control.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested
Intracellular staining (flow cytometry)	Routinely Tested
Isotype control	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-MOPC-21 immunoglobulins (Cat. No. 554680) is a suitable mouse IgG1κ isotype control for assessing the level of background staining on paraformaldehyde fixed/saponin-permeabilized mouse or human cells for flow cytometric analysis. Use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 μg mAb/1 million cells), (see image, right panel). For

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specific methodology, visit the protocols section of our website, or the chapter on intracellular staining in the Immune Function Handbook, which is posted on our web site at www.bdbiosciences.com. The intracellular cytokine staining technique and the use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554715	BD Cytotfix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555028	BD Cytotfix/Cytoperm Plus Kit (with BD GolgiPlug)	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. 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. (Biology: Flow cytometry)