Technical Data Sheet

Two-color Fluorescent Ig Isotype Cocktail with Human CD4+ PerCP-Cy™5.5

Product Information

Material Number:	**560798**
Size:	20 tests
Vol. per Test:	20 ul
Reactivity:	Reactivity: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

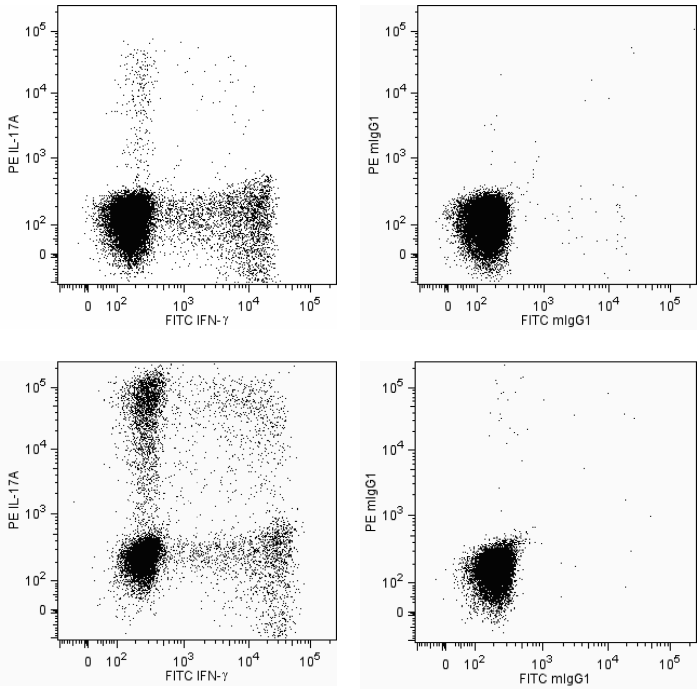
Description

Two-color Fluorescent Ig Isotype Cocktail

Containing the following:

Human CD4 PerCP-Cy5.5	**Clone: SK3**
Mouse mIgG1, κ FITC	**Clone: MOPC-21**
Mouse mIgG1, κ PE	**Clone: MOPC-21**

This multicolor cocktail contains a mixture of PerCP-Cy5.5-anti-Human CD4 antibody (Clone SK3) and FITC-Mouse IgG1, κ (MOPC-21) and PE-Mouse IgG1, κ (MOPC-21) isotype controls. This fluorescent Ig isotype control cocktail serves as a negative control for immunofluorescent staining and flow cytometric analysis using the BD Human Th1/Th17 Phenotyping Kit Cocktail (Component 51-9006617 of Cat. No. 560752). This phenotyping cocktail is comprised of PerCP-Cy5.5 anti-Human CD4, FITC-Mouse IgG1, κ anti-Human IFN-γ and PE-Mouse IgG1, κ anti-Human IL-17 antibodies. The BD Human Th1/Th17 Phenotyping Kit provides an easy-to-use three-color cocktail of fluorescent antibodies specific for human CD4, IFN-γ (for Th1) and IL-17A (for Th17) that enables researchers to identify and characterize the nature of T helper cell types by multicolor immunofluorescent staining and flow cytometric analysis.



Immunoglobulin isotype control staining of activated human CD4-positive T cells. *Human peripheral blood mononuclear cells (PBMC) (top panels) or PBMC pre-cultured under Th17 polarization conditions (bottom panels) were stimulated (5 h) with 50 ng/ml PMA (Sigma Cat. No. P-8139) and 1 µg/ml Ionomycin (Sigma Cat. No. I-0634) in the presence of BD GolgiStop™ (Cat. No. 554724). The cells were then fixed and permeabilized with BD Cytofix/Cytoperm™ (Cat. No. 554714) followed by staining with the HumanTh1/Th17 Phenotyping Kit (Cat. No. 560752) or the Two-color Fluorescent Ig Isotype Cocktail (Cat. No. 560798). The figure shows the staining of CD4-positive T cells for IFN-γ versus IL-17A (left panels) or for the matching Ig isotype controls (right panels). The two-color flow cytometric dot plots were derived from CD4-positive T cell-gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD LSR™ II flow cytometry system. See the protocol for stimulating, fixing, permeabilizing, and staining cells provided in the Technical Data Sheet for the Human Th1/Th17 Phenotyping Kit (Cat. No. 560752).*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Tested During Development

Recommended Assay Procedure:

The multicolor Ig isotype control cocktail is designed to control for nonspecific staining by fluorescent antibodies directed against cellular antigens found in target CD4+ T cells that have been fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Perm/Wash™ Buffer (Cat. No. 554723). Nonspecific staining by fluorescent antibodies may be due to nonspecific immunoglobulin- and/or fluorochrome-mediated binding to cellular molecules including Fc receptors for immunoglobulin. In addition, this multicolor Ig isotype cocktail can be used for negative control staining with other multicolor fluorescent antibody cocktails that contain PerCP-Cy5.5 anti-Human CD4 mixed with FITC- and PE-conjugated Mouse IgG1, κ antibodies. As such, the cocktail can provide negative controls for the immunofluorescent staining with fluorescent antibodies specific for other intracellular markers (eg, cytokines, chemokines, and/or transcription factors) expressed by CD4+ T cells that are prepared using Cytofix and Perm/Wash Buffers.

See the protocol for stimulating, fixing, permeabilizing, and staining cells provided in the Technical Data Sheet for the Human Th1/Th17 Phenotyping Kit Cocktail (Component 51-9006617 of Cat. No. 560752).

Suggested Companion Products

Catalog Number	Name	Size	Clone
560752	HumanTh1/Th17 Phenotyping Kit	50 tests	(none)
341654	PerCP-Cy5.5 Mouse anti-Human CD4	50 tests	SK3
554656	Stain Buffer (FBS)	500 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. 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.