Technical Data Sheet

PerCP-Cy™5.5 Mouse Anti-Human IL-4

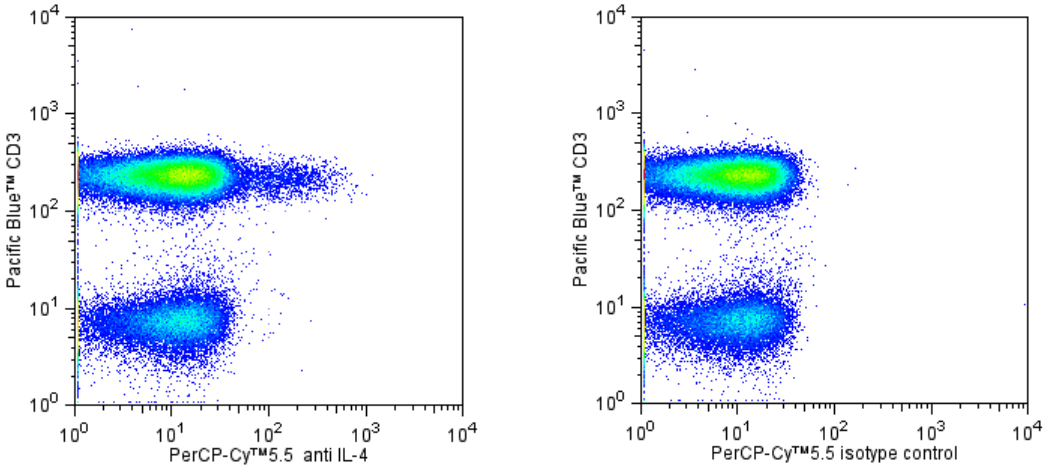
Product Information

Material Number:	**561234**
Alternate Name:	Interleukin-4; IL4; BCGF-1; BSF-1; Lymphocyte stimulatory factor 1
Size:	50 tests
Vol. per Test:	5 µl
Clone:	8D4-8
Immunogen:	Recombinant Human IL-4
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 8D4-8 monoclonal antibody reacts with human interleukin-4 (IL-4). The immunogen used to raise the 8D4-8 hybridoma was recombinant human IL-4. The 8D4-8 antibody binds to an epitope that is different than the epitope recognized by the MP4-25D2 antibody (Cat. No. 554485).

Clone 8D4-8 displays an increased amount of non-specific binding to dead cells when compared to the clone MP4-25D2. It is recommended to use a fixable viability dye in conjunction with this clone.



Expression of IL-4 by stimulated human lymphocytes. *Human peripheral blood mononuclear cells were stimulated for 6 h with Phorbol 12-Myristate 13-Acetate (PMA; Sigma, Cat. No. P-8139) and calcium ionophore A23187 (Sigma, Cat. No. C-9275) in the presence of GolgiStop™ Protein Transport Inhibitor (Cat. No. 554724). The cells were fixed using BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Perm/Wash™ Buffer (Cat. No. 554723). The cells were then stained with Pacific Blue™ Mouse Anti-Human CD3 (Cat No. 558117) and PerCP-Cy™5.5 Mouse anti-Human IL-4 antibody (Cat No. 561234, Left Panel) or PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control (Cat No. 550795, Right Panel) by using BD Biosciences Intracellular Cytokine Staining protocol. Two-color flow cytometric dot plots were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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 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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
550795	PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
558117	Pacific Blue™ Mouse Anti-Human CD3	0.1 mg	UCHT1

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. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. 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.
7. 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.
8. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
9. Cy is a trademark of Amersham Biosciences Limited.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Bird C, Wadhwa M, Thorpe R. Development of immunoassays for human interleukin 3 and interleukin 4, some of which discriminate between different recombinant DNA-derived molecules. *Cytokine.* 1991; 3(6):562-567. (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: Flow cytometry)

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