Technical Data Sheet

PE-Cy™7 Mouse Anti-Human IL-4

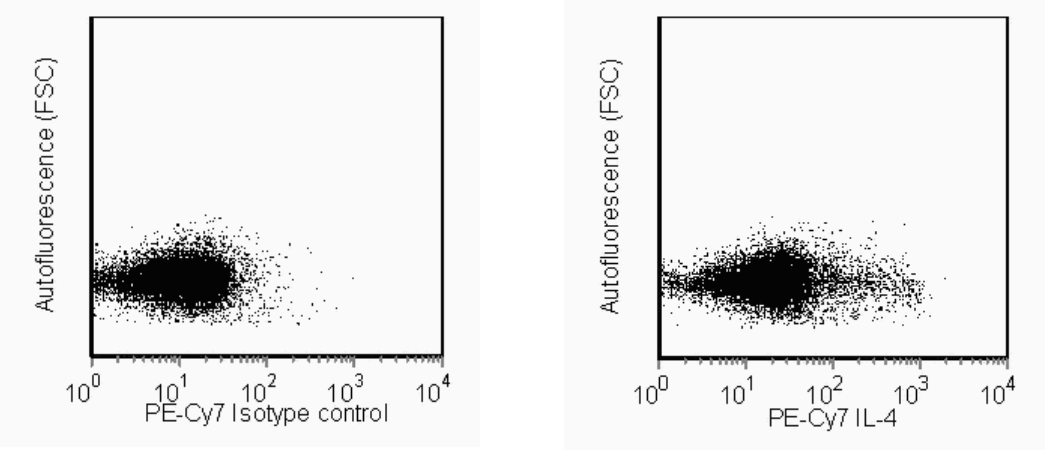
Product Information

Material Number:	**560672**
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.



Flow cytometric analysis for IL-4 in stimulated human peripheral blood mononuclear cells (PBMC).
Human PBMC were stimulated for at least 6 hours with 50 ng/mL PMA (Sigma-Aldrich Cat. No. P-8139) and 250 ng/mL calcium ionophore A23187 (Sigma-Aldrich Cat. No. C-9275) in the presence of 2 µM BD GolgiStop™ (Cat. No. 554724). Cells were then fixed and permeabilized using BD Cytofix/Cytoperm™ (Cat. No. 554714) followed by staining with either a PE-Cy™7 Mouse IgG1, κ isotype control (left panel) or with the PE-Cy™7 Mouse Anti-Human IL-4 antibody (right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry 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 PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: The 8D4-8 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-4 producing cells within mixed cell populations. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant human IL-4 (Cat. No. 554605) or (2) unlabeled 8D4-8 antibody (Cat. No. 554515), prior to staining.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557646	PE-Cy™7 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555062	HiCK-2 Human Cytokine Positive Control Cells	1.0 ml	(none)
554605	Recombinant Human IL-4	5 µg	(none)
554515	Purified Mouse Anti-Human IL-4	0.5 mg	8D4-8

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. 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.
7. 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).
8. Cy is a trademark of Amersham Biosciences Limited.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. 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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