Technical Data Sheet

PE Mouse Anti-Human IL-4

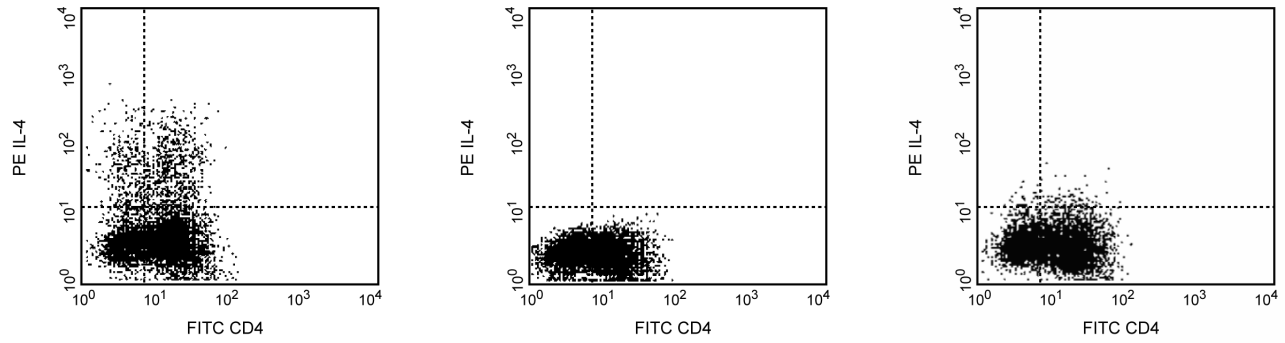
Product Information

Material Number:	**559333**
Size:	100 tests
Vol. per Test:	20 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 peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated with immobilized anti-human CD3 antibody (10 µg/ml for coating culture wells; Cat. No. 555329), soluble anti-human CD28 (2 µg/final concentration; Cat. No. 555725) recombinant human IL-2 (10 ng/ml final concentration; Cat. No. 554603) and recombinant human IL-4 (20 ng/ml final concentration; Cat. No. 554605) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and recombinant IL-4 for 3 days. Finally, the cells were harvested and stimulated for 4 hours with PMA (Sigma, Cat. #P-8139) and ionomycin (Sigma, Cat. #C-9275) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The cells were harvested, stained with FITC-anti-CD4 (Cat. No. 555346), fixed, permeabilized, and subsequently stained with 20 µl of PE-mouse anti-human IL-4 antibody (PE-8D4-8, Cat. No. 559333) by following Pharmingen's staining protocol (see image, left panel). To demonstrate specificity of staining, the binding of PE-8D4-8 antibody was blocked by preincubation of the antibody with recombinant human IL-4 (Cat. No. 554605; middle panel) or by preincubation of the fixed/permeabilized cells with unlabeled 8D4-8 antibody (10 µg, Cat. No. 556917; right panel) prior to staining. The quadrant markers for the bivariate dot plot were set based on the autofluorescence controls and verified using the recombinant cytokine and unlabeled antibody blocking controls.*

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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The PE-conjugated 8D4-8 antibody can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-4 producing cells within mixed cell populations (see image, left panel). A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is also available in a 100 Test Size formulation PE-MOPC-21 (Cat. No. 559320).

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the fluorochrome-conjugated 8D4-8 antibody with ligand (e.g., human IL-4; Cat. No. 554605) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled 8D4-8 antibody (Cat. No. 556917) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

Note: This pretitered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. Perm/Wash™ Buffer (Cat. No. 554723), included in the Cytofix/Cytoperm™ Plus Kits, does contain a permeabilization agent and is recommended for use with this antibody.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554605	Recombinant Human IL-4	5 µg	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
554603	Recombinant Human IL-2	10 µg	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
555062	HiCK-2 Human Cytokine Positive Control Cells	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. 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. 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. (Clone-specific)
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)

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