Technical Data Sheet

Alexa Fluor® 488 Mouse Anti-Human IL-4

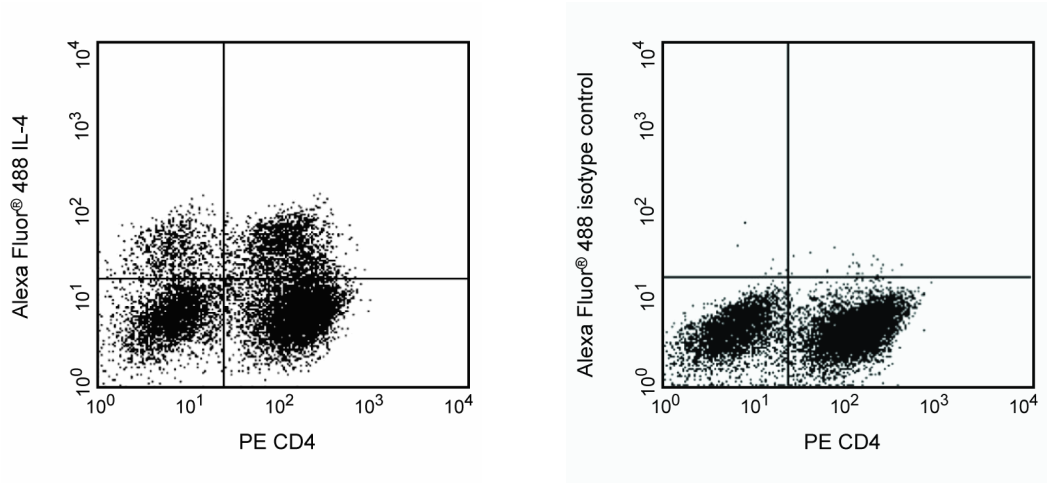
Product Information

Material Number:	**557990**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	8D4-8
Immunogen:	Recombinant Human IL-4
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤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. *Human peripheral blood mononuclear cells were stimulated with CD3 and CD28 in the presence of recombinant human IL-2 and IL-4. Following expansion in IL-2, IL-4 and stimulation with PMA and ionomycin the cells were stained with either PE anti-human CD4 and Alexa Fluor® 488 Mouse Anti-Human IL-4 (left panel) or immunoglobulin isotype control (Alexa Fluor® 488 Mouse IgG1 κ Isotype Control, clone MOPC-21, Cat. No. 557721) (right panel) by using Pharmingen's staining protocol. To demonstrate specificity of staining the binding of Alexa Fluor® 488 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant human IL-4 (data not shown) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype 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 to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

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. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde fixed/saponin permeabilized human cells is Alexa Fluor® 488 conjugated MOPC-21 (Cat. No. 557721).

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554605	Recombinant Human IL-4	5 µg	(none)
556917	Purified Mouse Anti-Human IL-4	0.1 mg	8D4-8
557721	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. 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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