Technical Data Sheet

Purified Mouse Anti-Human IL-4

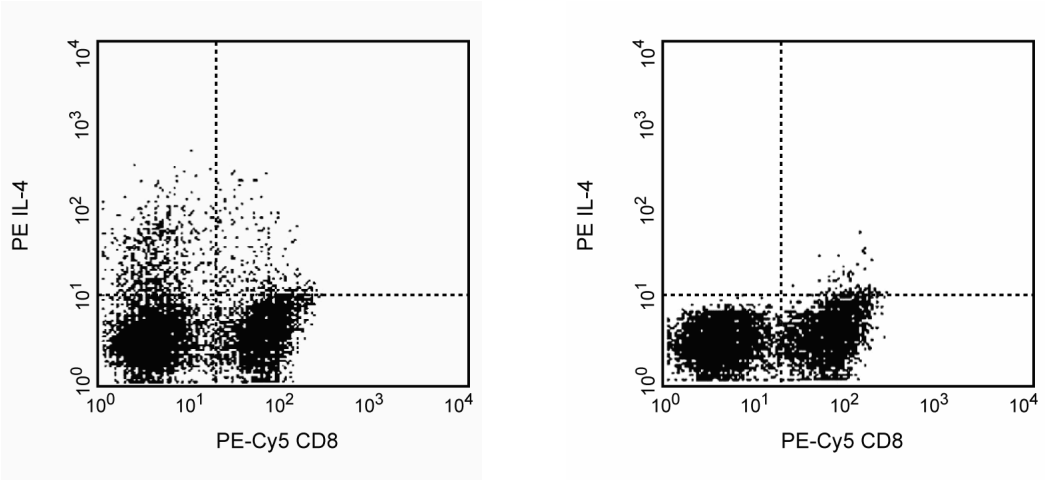
Product Information

Material Number:	**556917**
Size:	0.1 mg
Concentration:	0.5 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 (PBMC). *Human PBMC were stimulated with soluble anti-human CD3 antibody (1 µg/ml final concentration; Cat. No. 555329), recombinant human IL-2 (10 ng/ml final concentration; Cat. No. 554603) and recombinant human IL-4 (10 ng/ml final concentration; Cat. No. 554605) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and recombinant human IL-4 for 3 days. The cells were then harvested and stimulated for 6 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) and stained with PE-Cy5 - anti CD8 Finally the cells were fixed, permeabilized, and subsequently stained with 0.015 µg of PE-mouse anti-human IL-4 antibody (PE-8D4-8, Cat. No. 554516) by using the BD Pharmingen staining protocol (see image, left panel). To demonstrate specificity of staining, the binding of PE-8D4-8 antibody was blocked by preincubation of the PE-8D4-8 with recombinant human IL-4 (0.25 µg 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.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

ELISA Capture	Routinely Tested
Intracellular block/flow cytometry	Tested During Development

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Recommended Assay Procedure:

1. Blocking Control for Intracellular Staining: The purified 8D4-8 antibody (Cat. No. 556917) can be used as a blocking control to demonstrate specificity of IL-4 staining by conjugated-8D4-8 antibodies. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

2. ELISA Capture: The purified 8D4-8 antibody (Cat. No. 554515) is useful as a capture antibody for a sandwich ELISA for measuring human IL-4 protein levels. Purified 8D4-8 antibody can be paired with the biotinylated MP4-25D2 antibody (Cat. No. 554483) as the detecting antibody, with recombinant human IL-4 (Cat. No. 554605) as the standard. For testing IL-4 in serum or plasma, the OptEIA™ human IL-4 ELISA set (Cat. No. 555194) or OptEIA™ human IL-4 ELISA kit (Cat. No. 550614) are recommended.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554516	PE Mouse Anti-Human IL-4	0.1 mg	8D4-8
554515	Purified Mouse Anti-Human IL-4	0.5 mg	8D4-8
554483	Biotin Rat Anti-Human IL-4	0.5 mg	MP4-25D2
554605	Recombinant Human IL-4	5 µg	(none)
555194	Human IL-4 ELISA Set	20 plates	(none)
550614	Human IL-4 ELISA Kit II	2 plates	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
4. 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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