

Technical Data Sheet

APC Rat Anti-Human IL-4

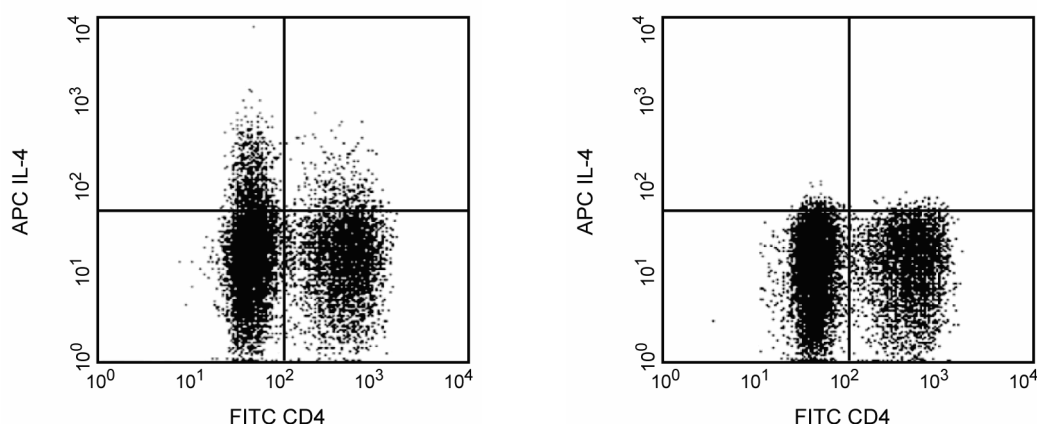
Product Information

Material Number:	554486
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	MP4-25D2
Immunogen:	Purified Recombinant Human IL-4
Isotype:	Rat IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MP4-25D2 antibody reacts with human interleukin-4 (IL-4). The immunogen used to generate the MP4-25D2 hybridoma was purified recombinant human IL-4. This is a neutralizing antibody. The MP4-25D2 antibody has been reported to cross react with IL-4 from rhesus monkeys. The use of the MP4-25D2 antibody for epitope mapping of human IL-4 has been described.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



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; Cat. No. 555329), recombinant human IL-2 (10 ng/ml, Cat. No. 554603) and recombinant human IL-4 (10 ng/ml; Cat. No. 554605) for 2 days. The cells were subsequently cultured in medium containing IL-2 and IL-4 for 3 days. Finally, the cells were harvested and stimulated for 6 h with PMA (Sigma) and calcium ionophore A23187 (Sigma) in the presence of BD GolgiStop™ (Cat. No. 554724). The cells were harvested, stained with FITC anti-CD4 (Cat. No. 555346) fixed, permeabilized, and subsequently stained with 0.05 µg of APC-rat anti-human IL-4 antibody (APC-MP4-25D2, Cat. No. 554486) by using BD Biosciences Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of APC-MP4-25D2 antibody was blocked by preincubation of the fixed/permeabilized cells with unlabelled MP4-25D2 antibody (2.5 µg, Cat. No. 554482; right panel) prior to staining. The quadrant markers for the bivariate dot plot were set based on the autofluorescence controls and verified using unlabelled antibody blocking controls. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe or red diode laser. These include the dual laser BD FACStarPLUS™, BD FACSVantage™ or BD FACSCalibur.™

Preparation and Storage

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

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed by gel filtration chromatography.

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Recommended Assay Procedure:

The APC-conjugated MP4-25D2 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). For optimal immunofluorescent staining for flow cytometric analysis, this antibody should be titrated ($\leq 0.20 \mu\text{g}$ mAb/million cells). 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.

A useful control for demonstrating specificity of staining is the following: pre-block the fixed/permeabilized cells with unlabelled MP4-25D2 antibody (Cat. No. 554482) prior to staining. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse and human cells is APC-R3-34 immunoglobulin (Cat. No. 554686); use at comparable concentrations to antibody of interest (eg, $\leq 0.20 \mu\text{g}$ mAb/1 million cells).

Suggested Companion Products

Catalog Number	Name	Size	Clone
555062	Hick-2 Cytokine Positive Control Cells	5x10 ⁶ cells	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554605	Recombinant Human IL-4	5 μg	(none)
554603	Recombinant Human IL-2	5 μg	(none)
555346	FITC Conjugated Mouse Anti-Human CD4	100 tests	RPA-T4
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
554686	APC Rat IgG1, κ Isotype Control	0.1 mg	R3-34

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
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.

References

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24.(Clone-specific: ELISA, Neutralization)
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Jung T, Schauer U, Rieger C, et al. Interleukin-4 and interleukin-5 are rarely co-expressed by human T cells. *Eur J Immunol.* 1995; 25(8):2413-2416.
(Clone-specific: Flow cytometry)
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: IC/FCM Block)
Ramanathan L, Ingram R, Sullivan L, et al. Immunochemical mapping of domains in human interleukin 4 recognized by neutralizing monoclonal antibodies. *Biochemistry.* 1993; 32(14):3549-3556.(Clone-specific: Neutralization)