

Technical Data Sheet

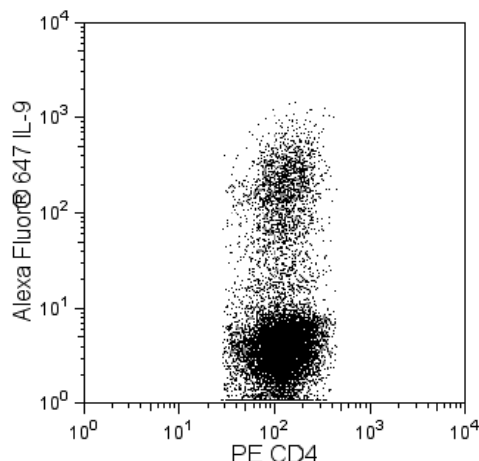
Alexa Fluor® 647 Mouse Anti-Human IL-9

Product Information

Material Number:	560813
Alternate Name:	IL9; IL-9; interleukin-9; HP40; P40
Size:	100 tests
Vol. per Test:	5 µl
Clone:	MH9A3
Immunogen:	Human IL-9 Recombinant Protein
Isotype:	Mouse (C57BL/6) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The MH9A3 monoclonal antibody specifically binds to human interleukin-9 (IL-9). Human IL-9 is a multifunctional cytokine and a member of the type I cytokine (hematopoietin) family that includes IL-2, IL-4, IL-7, IL-15 and IL-21. This cytokine is encoded by the *IL9* gene that is resident on chromosome 5q31.1. IL-9 is expressed by activated CD4-positive T helper cells, by some transformed T cells and by eosinophils, mast cells and neutrophils. IL-9 induces the proliferation, differentiation, and effector function of various cell types including T lymphocytes, B lymphocytes, mast cells, eosinophils, neutrophils, hematopoietic cells and epithelial cells. It potentiates the interleukin-4-induced IgM, IgG and IgE responses by human B lymphocytes. IL-9 has been implicated in human allergic disorders such as asthma and malignancies such as Hodgkin's disease. IL-9 exerts its biological activities through binding to the surface IL-9 receptor (IL-9R) complex comprised of the IL-9R alpha subunit (IL-9Rα; CD129) and the common cytokine receptor gamma subunit (γc; CD132). IL-9 signaling through its receptor includes activation of the Janus kinases 1 and 3 (JAK1 and JAK3) and activation of Signal transducer and activator of transcription 1, 3 and 5 factors (STAT1, STAT3 and STAT5).



Flow cytometric analysis of IL-9 in stimulated human CD4-positive T cells. Human peripheral blood mononuclear cells were stimulated in a tissue culture plate coated with NA/LE Mouse Anti-Human CD3 (Cat. No. 555329; 10 µg/ml, coated overnight at 4°C) and soluble NA/LE Mouse Anti-Human CD28 (Cat. No. 555725; 1 µg/ml) antibodies plus recombinant Human IL-2 (Cat. No. 554603; 10 ng/ml), IL-4 (Cat. No. 554605; 50 ng/ml), and TGF-β (Cat. No. 356039; 10 ng/ml) proteins and NA/LE Mouse Anti-Human IFN-γ (Cat. No. 554698; 10 µg/ml) antibody for 5 days. The cells were harvested and restimulated with PMA (Sigma P8139; 50 ng/ml) and ionomycin (Sigma I9657; 1 µg/ml) in the presence of BD GolgiStop™ Protein Transport Inhibitor (Cat. No. 554724) for 5 hours. The cells were then fixed and permeabilized using the BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (Cat. No. 554714) followed by staining with Alexa Fluor® 647 Mouse Anti-Human IL-9 (Cat. No. 560813) and PE Mouse Anti-Human CD4 (Cat. No. 555347) antibodies. Two-color flow cytometric dot plots showing correlated expression patterns of CD4 versus IL-9 were derived from CD4-positive cell gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed on a BD LSRII™ System.

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Preparation and Storage

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

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21
555347	PE Mouse Anti-Human CD4	100 tests	RPA-T4
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554655	Fixation Buffer	100 ml	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit	250 tests	(none)
554603	Recombinant Human IL-2	10 µg	(none)
554605	Recombinant Human IL-4	5 µg	(none)
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
554698	Purified NA/LE Mouse Anti-Human IFN-γ	0.5 mg	B27
354039	Transforming Growth Factor-beta (TGF-β), human natural, 1 µg	10 tests	hTGF- beta
356039	Transforming Growth Factor-β (TGF-β), human natural, 1 X 5 µg	NA	(none)
356040	Transforming Growth Factor-β (TGF-β), human natural, 5 X 2 µg	NA	(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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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