Technical Data Sheet

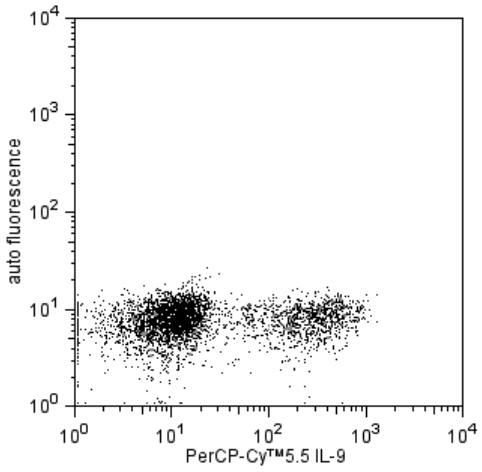
PerCP-Cy™5.5 Mouse Anti-Human IL-9

Product Information

Material Number:	**561461**
Alternate Name:	IL9; IL-9; interleukin-9; HP40; P40
Size:	50 tests
Vol. per Test:	5 µl
Clone:	MH9A3
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 expressed 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/Permeablization Kit (Cat. No. 554714) followed by staining with PerCP-Cy™5.5 Mouse Anti-Human IL-9 (Cat. No. 561461) and Mouse Anti-Human CD4 (Cat. No. 555347) antibodies. Two-color flow cytometric dot plots showing IL-9 versus autofluorescence (PE channel) were derived from CD4 positive gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed on a BD LSRII™ System.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554656	Stain Buffer (FBS)	500 ml	(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
554603	Recombinant Human IL-2	10 µg	(none)
356039	Transforming Growth Factor-b (TGF-b), human natural, 1 X 5 µg	NA	(none)
554605	Recombinant Human IL-4	5 µg	(none)
554698	Purified NA/LE Mouse Anti-Human IFN-γ	0.5 mg	B27

Product Notices

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
2. 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).
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
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. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
9. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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References

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