Technical Data Sheet

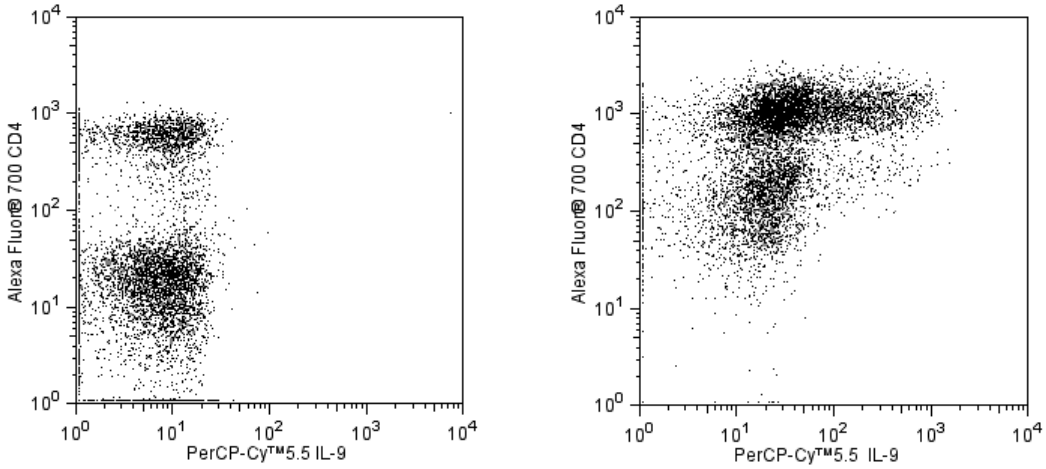
PerCP-Cy™5.5 Armenian Hamster Anti-Mouse IL-9

Product Information

Material Number:	**561492**
Alternate Name:	IL-9; Interleukin-9; MEA; P40; T-cell growth factor P40; TCGF III
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	D9302C12
Immunogen:	Mouse IL-9 Recombinant Protein
Isotype:	Armenian Hamster IgG2, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The D9302C12 monoclonal antibody specifically binds to the multifunctional mouse cytokine, Interleukin-9 (IL-9). IL-9 is a 126 amino acid-long glycoprotein that is produced by various subsets of activated CD4+ T cells. IL-9 acts on target cells by binding to and signaling through the heterodimeric IL-9 receptor (IL-9R) complex that is comprised of transmembrane IL-9 receptor alpha (IL-9Rα) and common gamma chain (γc) subunits. IL-9 can promote the survival, growth, proliferation and/or differentiation of various cell types including thymocytes, T cells, B cells, mast cells, and hematopoietic progenitor cells. IL-9 can augment IL-4-induced IgE and IgG1 production from lipopolysaccharide-primed mouse B cells and induce granzyme and high-affinity IgE receptor gene expression by mouse T helper cell clones and mast cell lines. IL-9 plays an important role *in vivo* in helminth elimination. The D9302C12 antibody neutralizes mouse IL-9 bioactivity.



Multicolor flow cytometric analysis of IL-9 expression by unstimulated and activated mouse spleen cells. *Mouse spleen cells were either unstimulated (Left Panel) or stimulated in a tissue culture plate coated with Anti-Mouse CD3e and soluble Anti-Mouse CD28 antibodies along with Recombinant Mouse IL-2, IL-4, and TGF-β proteins and Anti-Mouse IFN-γ antibody for 4 days. On day 4 the cells were harvested and restimulated with Phorbol 12-Myristate 13-Acetate (PMA; Sigma P-8139) plus Ionomycin (Sigma; I-0634) in the presence of BD GolgiStop™ Protein Transport Inhibitor for 5 hours (Right Panel). The cells were then fixed and permeabilized using a BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit followed by staining with PerCP-Cy™5.5 Armenian Hamster Anti-Mouse IL-9 (Cat. No. 561492) and Alexa Fluor® 700 Rat Anti-Mouse CD4 (Cat. No. 561025/557956). Two-color flow cytometric dot plots showing the correlated expression patterns of CD4 versus IL-9 were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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 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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
557870	Fix Buffer I	250 ml	(none)
561025	Alexa Fluor® 700 Rat Anti-Mouse CD4	25 µg	RM4-5
557956	Alexa Fluor® 700 Rat Anti-Mouse CD4	0.1 mg	RM4-5
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
550069	Recombinant Mouse IL-2	20 µg	(none)
550067	Recombinant Mouse IL-4	10 µg	(none)
356039	Transforming Growth Factor-b (TGF-b), human natural, 1 X 5 µg	NA	(none)
554408	Purified NA/LE Rat Anti-Mouse IFN-γ	0.5 mg	XMG1.2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. 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.
4. 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.
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. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Van Snick J, Cayphas S, Vink A, Uyttenhove C, Coulie PG, Rubira MR, Simpson RJ. Purification and NH2-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas. *Proc Natl Acad Sci U S A.* 1986; 83(24):9679-9683. (Biology)

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