

Technical Data Sheet

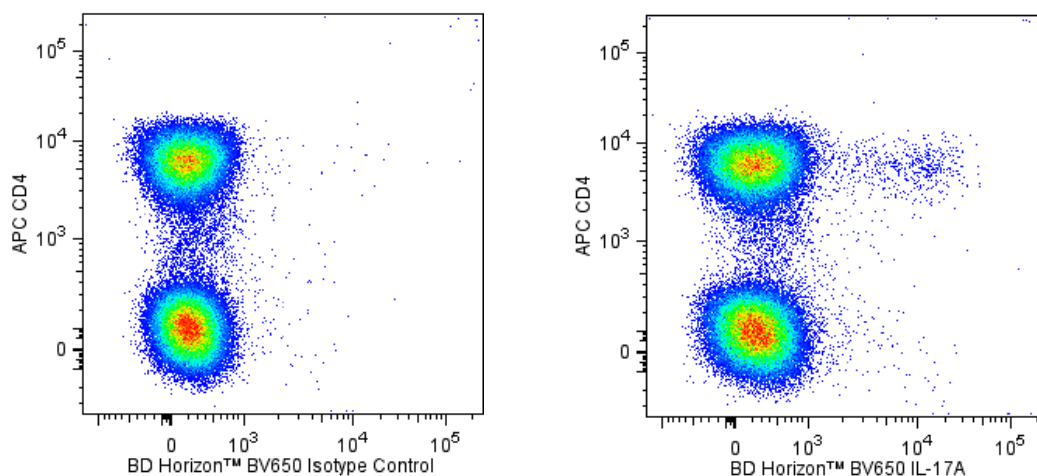
BV650 Mouse Anti-Human IL-17A**Product Information**

Material Number:	563746
Alternate Name:	IL-17; CTLA8; Cytotoxic T-lymphocyte-associated serine esterase 8
Size:	50 tests
Vol. per Test:	5 µl
Clone:	N49-653
Immunogen:	Human IL-17A Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Human IL-17A, also known as IL-17, is a proinflammatory cytokine that is encoded by the IL17A gene in chromosome 6. IL-17A is produced as a disulfide-linked homodimer comprised of two mature 136-amino acid polypeptides. It is a member of the IL-17 family of structurally related cytokines, designated IL-17A through IL-17F. Activated memory T cells, especially Th17 cells (specialized IL-17A-producing CD4+ T cells distinct from Th1 and Th2 cells) produce IL-17 and provide protective immunity against pathogens. Activated CD8+ T cells, γδT cells, NK cells and neutrophils can also be activated to produce IL-17A. IL-17A binds to and exerts its biological activity through IL-17 receptors (IL-17R) that are expressed by a variety of target cells including fibroblasts, epithelial and endothelial cells, monocytes/macrophages and mast cells. The ubiquitous IL-17R expression pattern may explain the broad tissue responsiveness to IL-17. IL-17 induces stromal cells to secrete cytokines and chemokines involved in inflammatory and hematopoietic processes. For example, IL-17 induces fibroblasts to produce IL-6, IL-8, G-CSF and express increased surface ICAM-1. The N49-653 antibody reacts with human IL-17A.

The antibody was conjugated to BD Horizon™ BV650 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon™ BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon™ BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg, 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



Two-color flow cytometric analysis of IL-17A expression in stimulated human peripheral blood lymphocytes. Human peripheral blood mononuclear cells were stimulated for 5 hours with Phorbol 12-Myristate 13-Acetate (Sigma P-8139; 50 ng/ml final concentration) and Ionomycin (Sigma I-0634; 1 µg/ml final concentration) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested, washed with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (Cat. No. 554722). The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with APC Mouse Anti-Human CD4 antibody (Cat. No. 555349/561840/561841) and either BD Horizon™ BV650 Mouse IgG1 κ Isotype Control (Cat. No. 563231; Left Panel) or BD Horizon™ BV650 Mouse Anti-Human IL-17A antibody (Cat. No. 563746; Right Panel). Two-color flow cytometric dot plots show the correlated expression patterns of IL-17A (or Ig Isotype control staining) versus CD4 for gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

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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 BD Horizon™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563231	BV650 Mouse IgG1, k Isotype Control	50 µg	X40
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
555349	APC Mouse Anti-Human CD4	100 tests	RPA-T4
561841	APC Mouse Anti-Human CD4	500 tests	RPA-T4
561840	APC Mouse Anti-Human CD4	25 tests	RPA-T4

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. An isotype control should be used at the same concentration as the antibody of interest.
3. Brilliant Violet™ 650 is a trademark of Sirigen.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharminen/protocols for technical protocols.

References

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