Technical Data Sheet

BV421 Mouse Anti-Human IL-3Rα (CD123)

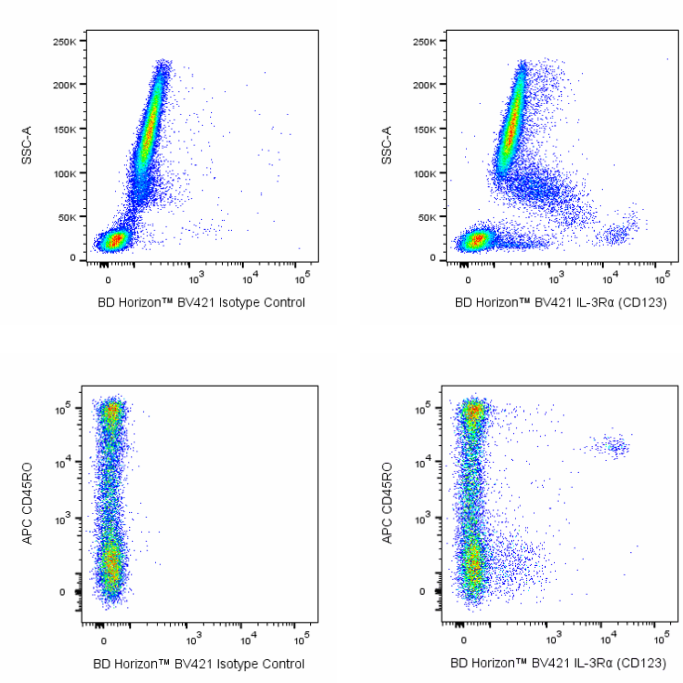
Product Information

Material Number:	**567279**
Alternate Name:	IL3RA; IL3R; IL-3R-alpha; IL-3RA
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	6H6
Immunogen:	Human IL3RA Transfected Cell Line
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 6H6 monoclonal antibody specifically recognizes the Interleukin-3 receptor alpha chain (IL-3Ra) which is also known as CD123. IL-3Ra (CD123) is a ~70 kDa type I transmembrane glycoprotein that is encoded by *IL3RA* (interleukin 3 receptor subunit alpha) which belongs to the type I cytokine receptor family within the immunoglobulin gene superfamily. This receptor chain consists of an extracellular region that contains an immunoglobulin-like N-terminal domain (NTD) with a fibronectin type III (FnIII) fold followed by two more FnIII domains that form the cytokine receptor module (CRM), a transmembrane region, and an intracellular tail. IL-3Ra (CD123) binds IL-3 specifically and with low affinity. IL-3Ra (CD123) forms a high-affinity signaling receptor for IL-3 (IL-3R) with the ß common chain (ßc; also known as, CD131) that is shared with the heterodimeric IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors. IL-3Ra (CD123) is variably expressed on certain hematopoietic progenitor cells, basophils, eosinophils, mast cells, monocytes, macrophages, dendritic cells, megakaryocytes, and on some B cells, endothelial cells, and leukemia cells. Bound IL-3 can signal through IL-3R to promote the activation, proliferation, differentiation, and viability of these cells. Amongst monoclonal antibodies specific for human IL-3Ra (CD123), the 6H6 and 9F5 antibodies do not block IL-3 binding to the IL-3R whereas the 7G3 antibody does block IL-3 binding to its receptor in a dose-dependent manner.

The antibody was conjugated to BD Horizon BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max near 407 nm and Em Max near 421 nm, BD Horizon BV421 can be excited by the violet laser (405 nm) and detected with a 450/50 nm filter. BD Horizon BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates. Due to nearly identical excitation and emission properties but different spillover characteristics, BD Horizon BV421, Pacific Blue™, and BD Horizon V450 cannot be used simultaneously.



Multiparameter flow cytometric analysis of IL-3Rα (CD123) expression on human peripheral blood leucocyte populations. *Human whole blood was stained with APC Mouse Anti-Human CD45RO (Cat No. 559865/ 560899; Bottom Plots) and with either BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat No. 562438; Left Plots) or BD Horizon™ BV421 Mouse Anti-Human IL-3Rα (CD123) antibody (Cat No. 567279/567280; Right Plots). Erythrocytes were lysed with BD FACS™ Lysing Solution (Cat No. 349202). Upper Plots: Bivariate pseudocolor density plots showing the correlated expression of IL-3Rα (CD123) [or Ig Isotype control staining] versus side light-scatter (SSC-A) signals were derived from gated events with the forward and side light-scatter characteristics of intact human leucocytes. Lower Plots: Bivariate pseudocolor density plots showing the correlated expression of IL-3Rα (CD123) [or Ig Isotype control staining] versus CD45RO were derived from gated events with the forward and side light-scatter characteristics of intact human lymphocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ software.*

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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 to the dye under optimum conditions and unconjugated antibody and free dye were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and CompBead to ensure that BD Comp beads are appropriate for your specific cellular application.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

Catalog Number	Name	Size	Clone
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
349202	Lysing Solution 10X Concentrate	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
567280	BV421 Mouse Anti-Human IL-3Rα (CD123)	25 Tests	6H6
562438	BV421 Mouse IgG1, k Isotype Control	50 µg	X40
559865	APC Mouse Anti-Human CD45RO	100 Tests	UCHL1
560899	APC Mouse Anti-Human CD45RO	25 Tests	UCHL1
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(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. An isotype control should be used at the same concentration as the antibody of interest.
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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. BD Horizon Brilliant Violet 421 is covered by one or more of the following US patents: 8,158,444; 8,362,193; 8,575,303; 8,354,239.
8. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
9. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
10. Pacific Blue™ is a trademark of Life Technologies Corporation.
11. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

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