Technical Data Sheet

BV421 Rat Anti-Mouse CD8a

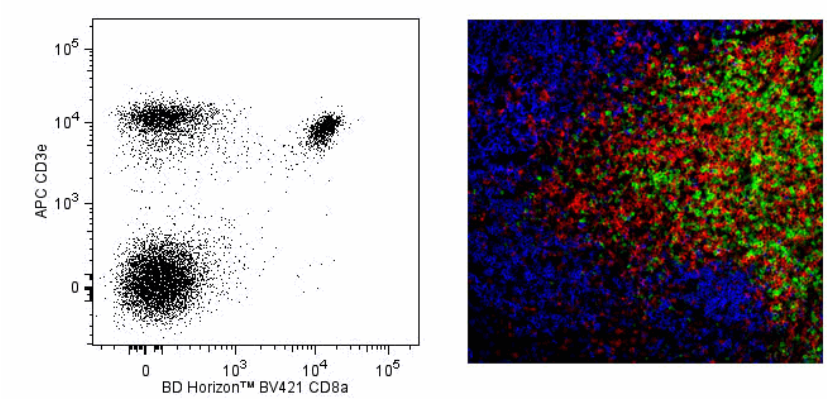
Product Information

Material Number:	**563898**
Alternate Name:	Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	53-6.7
Immunogen:	Mouse Spleen Cells or Thymocyte Membranes
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 53-6.7 monoclonal antibody specifically binds to the 38 kDa α and 34 kDa α' chains of the CD8 differentiation antigen (Ly-2 or Lyt-2) of all mouse strains tested. The CD8 α and α' chains (CD8a) form heterodimers with the CD8 β chain (CD8b, Ly-3, or Lyt-3) on the surface of most thymocytes. A subpopulation of mature T lymphocytes (i.e., MHC class I-restricted T cells, including most T suppressor/cytotoxic cells) expresses almost exclusively the CD8 αβ heterodimer. Subsets of γδ TCR-bearing T cells, intestinal intrapithelial lymphocytes, and dendritic cells express CD8a without CD8b. It has been suggested that the expression of the CD8a/CD8b heterodimer is restricted to T lymphocytes which matured in the thymus or in an extrathymic environment that had been influenced by thymus-initiated neuroendocrine signals. CD8 is an antigen coreceptor on the T-cell surface which interacts with MHC class I molecules on antigen-presenting cells or epithelial cells. It participates in T-cell activation through its association with the T-cell receptor complex and protein tyrosine kinase lck (p56 [lck]). The CD8 α and α' chains arise from alternatively spliced messengers of a single *CD8a* gene. The longer α form associates with p56 [lck] via a CXCP motif in its cytoplasmic domain, which it shares with CD4, but not with CD8b. The truncated α' chain is unable to associate with p56 [lck], and it may function to attenuate the CD8-mediated costimulatory signal during intrathymic T-cell maturation. In vivo and in vitro treatment with 53-6.7 mAb has reportedly been effective at depleting CD8+ peripheral T lymphocytes. The 53-6.7 antibody has also been reported to cross-react with CD8 α- and α'-like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad, *Bufo regularis*.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.



Two-color flow cytometric analysis of CD8a expression on mouse splenocytes (left panel). *Mouse splenic leucocytes were stained with APC Hamster Anti-Mouse CD3e (Cat. No. 561826/553066) and BD Horizon™ BV421 Rat Anti-Mouse CD8a (Cat. No. 563898) antibodies. The two-color fluorescence dot plot shows the correlated expression patterns of CD8a versus CD3e for gated events with the forward and side light-scatter characteristic of viable splenic leucocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.* ***Immunofluorescent analysis of CD8a expression by cells within C57BL/6 mouse spleen (right panel).*** *A mouse spleen cryosection (5 µm) was fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), blocked with 5% goat serum and 1% BSA diluted in 1x PBS, and stained with BD Horizon™ BV421 Rat Anti-Mouse CD8a antibody (Cat. No. 563898, pseudo-colored green), Alexa Fluor® 488 Rat Anti-Mouse CD4 antibody (Cat. No. 557667, pseudo-colored red), and Alexa Fluor® 647 Rat Anti-Mouse CD45R/B220 antibody (Cat. No. 557683, pseudo-colored blue). Images were captured on a standard epifluorescence microscope. Original magnification, 20x.*

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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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

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).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
562602	BV421 Rat IgG2a, κ Isotype Control	50 µg	R35-95
553066	APC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
561826	APC Hamster Anti-Mouse CD3e	25 µg	145-2C11
555899	Lysing Buffer	100 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
564907	DAPI Solution	1 mg	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
554655	Fixation Buffer	100 mL	(none)
557667	Alexa Fluor® 488 Rat Anti-Mouse CD4	0.1 mg	RM4-5
557683	Alexa Fluor® 647 Rat Anti-Mouse CD45R	0.1 mg	RA3-6B2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. 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.
9. 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.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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