

## Technical Data Sheet

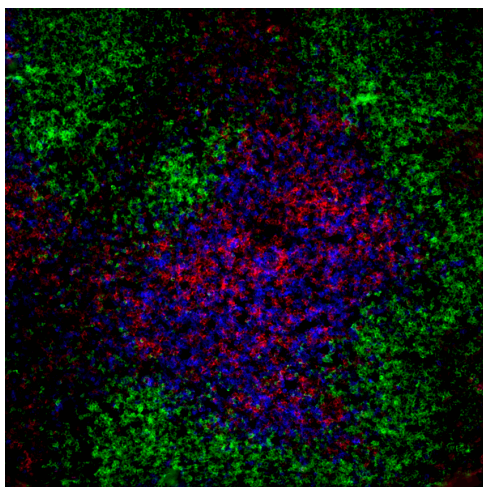
## Alexa Fluor® 647 Rat Anti-Mouse CD8a

## Product Information

Material Number:	557682
Alternate Name:	CD8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	53-6.7
Immunogen:	Mouse Spleen Cells or Thymocyte Membranes
Isotype:	Rat (LOU) IgG2a, $\kappa$
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

## Description

The 53-6.7 monoclonal antibody specifically binds to the 38 kDa  $\alpha$  and 34 kDa  $\alpha'$  chains of the CD8 differentiation antigen (Ly-2 or Lyt-2) of all mouse strains tested. The CD8  $\alpha$  and  $\alpha'$  chains (CD8a) form heterodimers with the CD8  $\beta$  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  $\alpha\beta$  heterodimer. Subsets of  $\gamma\delta$  TCR-bearing T cells, intestinal intraepithelial 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  $\alpha$  and  $\alpha'$  chains arise from alternatively spliced messengers of a single *CD8a* gene. The longer  $\alpha$  form associates with p56 [lck] via a CXCP motif in its cytoplasmic domain, which it shares with CD4, but not with CD8b. The truncated  $\alpha'$  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<sup>+</sup> peripheral T lymphocytes. The 53-6.7 antibody has also been reported to cross-react with CD8  $\alpha$ - and  $\alpha'$ -like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad, *Bufo regularis*.



**Immunohistofluorescent analysis of CD8a expression by cells within C57BL/6 mouse spleen.** A mouse spleen cryosection (5  $\mu$ m) was fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655), blocked with 5% goat serum and 1% BSA diluted in 1x PBS, and stained with Alexa Fluor® 647 Rat-anti-Mouse CD8a (Cat. No. 557682, pseudo-colored red), BD Horizon™ BV421 Rat Anti-Mouse CD45R/B220 antibody (Cat. No. 562922, pseudo-colored green), BD Horizon™ BV480 Rat Anti-Mouse CD4 antibody (Cat. No. 565634, pseudo-colored blue). Images were captured on a standard epifluorescence microscope. Original magnification, 20x.

## 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 Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

## Application Notes

## Application

Flow cytometry	Routinely Tested
Immunofluorescence	Tested During Development

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557682 Rev. 3



## Suggested Companion Products

Catalog Number	Name	Size	Clone
557690	Alexa Fluor® 647 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
564907	DAPI Solution	1 mg	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
554655	Fixation Buffer	100 mL	(none)
562922	BV421 Rat Anti-Mouse CD45R/B220	50 µg	RA3-6B2
565634	BV480 Rat Anti-Mouse CD4	50 µg	RM4-5

## 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

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