

## Technical Data Sheet

## R718 Rat Anti-Mouse CD8a

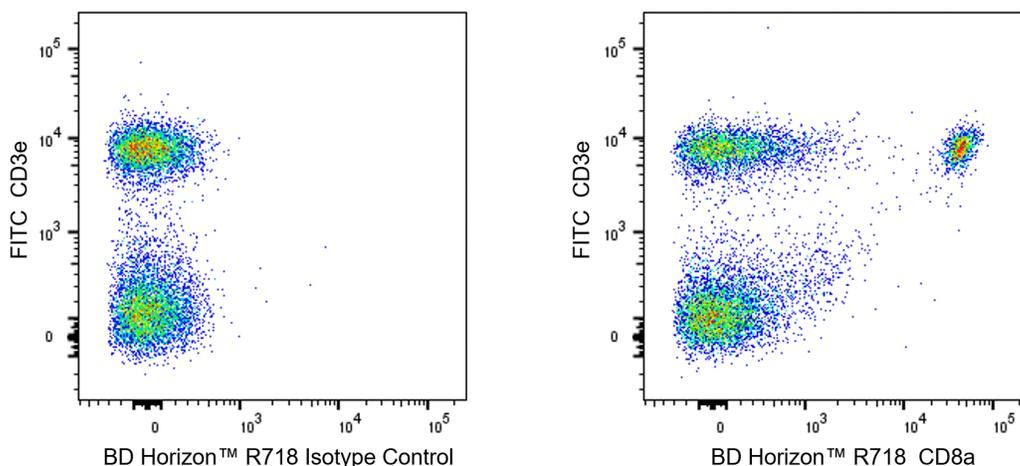
## Product Information

<b>Material Number:</b>	566985
<b>Alternate Name:</b>	Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
<b>Size:</b>	50 µg
<b>Concentration:</b>	0.2 mg/ml
<b>Clone:</b>	53-6.7
<b>Immunogen:</b>	Mouse Spleen Cells or Thymocyte Membranes
<b>Isotype:</b>	Rat (LOU) IgG2a, κ
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤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+ 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*.

The antibody was conjugated to BD Horizon Red 718, which has been developed exclusively for BD Biosciences as a better alternative to Alexa Fluor® 700. BD Horizon Red 718 can be excited by the red laser (628 – 640 nm) and, with an Em Max around 718 nm, it can be detected using a 730/45 nm filter. Due to similar excitation and emission properties, we do not recommend using R718 in combination with APC-R700 or Alexa Fluor® 700.



**Two-color flow cytometric analysis of CD8a expression on mouse splenocytes.** Mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with FITC Hamster Anti-Mouse CD3e antibody (Cat. No. 553061/553062) and either BD Horizon™ R718 Rat IgG2a, κ Isotype Control (Cat. No. 566941; Left Plot) or BD Horizon™ R718 Rat Anti-Mouse CD8a (Cat. No. 566985; Right Plot) at 0.5 µg/test. A bivariate pseudocolor density plot showing the correlated expression of CD8a (or Ig Isotype control staining) versus CD3e was derived from gated events with the forward and side light-scatter characteristics of viable leucocytes. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Flow Cytometer System and FlowJo™ software. Data shown on this Technical Data Sheet are not lot specific.

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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 unreacted dye was 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 BD 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 BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.

### Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555899	Lysing Buffer	100 mL	(none)
553061	FITC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
553062	FITC Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
561827	FITC Hamster Anti-Mouse CD3e	25 µg	145-2C11
566941	R718 Rat IgG2a, κ Isotype Control	50 µg	R35-95

### 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. Alexa Fluor® is a registered trademark of Life Technologies Corporation.
5. Please refer to <http://regdocs.bd.com> to access safety data sheets (SDS).
6. Please refer to [www.bdbiosciences.com/us/s/resources](http://www.bdbiosciences.com/us/s/resources) for technical protocols.
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