

# Technical Data Sheet

## BUV563 Rat Anti-Mouse CD8a

### Product Information

|                    |   |
|--------------------|---|
| Material Number:   | 748535  |
| Size:              | 50 µg   |
| Clone:             | 53-6.7  |
| Alternative Name:  | Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B     |
| Reactivity:        | Mouse (Tested in Development)                             |
| Isotype:           | Rat LOU, also known as Louvain, LOU/C, LOU/M IgG2a, κ     |
| Immunogen:         | Mouse Spleen Cells or Thymocyte Membranes                 |
| Application:       | Flow cytometry (Qualified)                                |
| Concentration:     | 0.2 mg/ml   |
| Entrez Gene ID:    | 12525   |
| Storage Buffer:    | Aqueous buffered solution containing ≤0.09% sodium azide. |
| Regulatory Status: | RUO   |

### 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™ BUV563 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome of BD Horizon BUV395 which has an Ex Max of 348 nm and an acceptor dye. The tandem has an Em Max at 563 nm. BD Horizon BUV563 can be excited by the 355 nm ultraviolet laser. On instruments with a 561 nm Yellow-Green laser, the recommended bandpass filter is 585/15 nm with a 535 nm long pass to minimize laser light leakage. When BD Horizon BUV563 is used with an instrument that does not have a 561 nm laser, a 560/40 nm filter with a 535 nm long pass may be more optimal. Due to the excitation and emission characteristics of the acceptor dye, there may be spillover into the PE and PE-CF594 detectors. However, the spillover can be corrected through compensation as with any other dye combination.

### Preparation and Storage Section

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 BUV563 under optimal conditions that minimize unconjugated dye and antibody.

### Recommended Assay Procedure

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes (including BD OptiBuild Brilliant reagents) 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 |
|----------------|--|------------|-------|
| 612922         | BUV563 Rat IgG2a, $\kappa$ Isotype Ctrl R35-95 RUO               | 50 $\mu$ g |       |
| 554656         | Stain Buffer (FBS) RUO   | 500 mL     |       |
| 554657         | Stain Buffer (BSA) RUO   | 500 mL     |       |
| 563794         | Brilliant Stain Buffer RUO                                       | 100 Tests  |       |
| 555899         | Lysing Buffer RUO  | 100 mL     |       |
| 553141         | Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) 2.4G2 RUO | 0.1 mg     |       |
| 565804         | Red Nucleic Acid Stain RUO                                       | 0.5 mL     |       |

## Product Notices

1. This antibody was developed for use in flow cytometry.
2. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.
3. Researchers should determine the optimal concentration of this reagent for their individual applications.
4. An isotype control should be used at the same concentration as the antibody of interest.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [wwwbdbiosciences.com/colors](http://wwwbdbiosciences.com/colors).
7. Please refer to [wwwbdbiosciences.com/us/s/resources](http://wwwbdbiosciences.com/us/s/resources) for technical protocols.
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.

## References

- Fujiura Y, Kawaguchi M, Kondo Y, et al. Development of CD8 alpha alpha+ intestinal intraepithelial T cells in beta 2-microglobulin- and/or TAP1-deficient mice. *J Immunol.* 1996; 156(8):2710-2715. (Clone-specific: Flow cytometry).
- Hathcock KS. T cell depletion by cytotoxic elimination. *Curr Protoc Immunol.* 1991; 1:3.4.1-3.4.3. (Clone-specific: Flow cytometry).
- Kruisbeek AM, Shevach EM. Proliferative assays for T cell function. *Curr Protoc Immunol.* 2004; 3:3.12.1-3.12.14. (Clone-specific: Flow cytometry).
- Ledbetter JA, Herzenberg LA. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol Rev.* 1979; 47:63-90. (Immunogen: Flow cytometry).
- Ledbetter JA, Rouse RV, Micklem HS, Herzenberg LA. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. *J Exp Med.* 1980; 152(2):280-295. (Immunogen: Flow cytometry).
- Ledbetter JA, Seaman WE, Tsu TT, Herzenberg LA. Lyt-2 and lyt-3 antigens are on two different polypeptide subunits linked by disulfide bonds. Relationship of subunits to T cell cytolytic activity. *J Exp Med.* 1981; 153(6):1503-1516. (Clone-specific: Flow cytometry).
- Leishman AJ, Naidenko OV, Attinger A, et al. T cell responses modulated through interaction between CD8alphaalpha and the nonclassical MHC class I molecule, TL. *Science.* 2001; 294(5548):1848-1849. (Clone-specific: Flow cytometry).
- MacDonald HR, Schreyer M, Howe RC, Bron C. Selective expression of CD8 alpha (Ly-2) subunit on activated thymic gamma/delta cells. *Eur J Immunol.* 1990; 20(4):927-930. (Biology: Flow cytometry).
- Nakayama K, Nakayama K, Negishi I, et al. Requirement for CD8 beta chain in positive selection of CD8-lineage T cells. *Science.* 1994; 263(5150):1131-1133. (Biology: Flow cytometry).
- Sydora BC, Brossay L, Hagenbaugh A, Kronenberg M, Cheroutre H. TAP-independent selection of CD8+ intestinal intraepithelial lymphocytes. *J Immunol.* 1996; 156(11):4209-4216. (Clone-specific: Flow cytometry).
- Takahashi K, Nakata M, Tanaka T, et al. CD4 and CD8 regulate interleukin 2 responses of T cells. *Proc Natl Acad Sci U S A.* 1992; 89(12):5557-5561. (Clone-specific: Flow cytometry).
- van Ewijk W, van Soest PL, van den Engh GJ. Fluorescence analysis and anatomic distribution of mouse T lymphocyte subsets defined by monoclonal antibodies to the antigens Thy-1, Lyt-1, Lyt-2, and T-200. *J Immunol.* 1981; 127(6):2594-2604. (Clone-specific: Flow cytometry).
- Vremec D, Zorbas M, Scollay R, et al. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *J Exp Med.* 1992; 176(1):47-58. (Clone-specific: Flow cytometry).
- Wang J, Klein JR. Thymus-neuroendocrine interactions in extrathymic T cell development. *Science.* 1994; 265(5180):1860-1862. (Biology: Flow cytometry).
- Wu L, Vremec D, Ardavin C, et al. Mouse thymus dendritic cells: kinetics of development and changes in surface markers during maturation. *Eur J Immunol.* 1995; 25(2):418-425. (Biology: Flow cytometry).

## BD Biosciences

[bdbiosciences.com](http://bdbiosciences.com)

United States  
877.232.8995

Canada  
888.268.5430

Europe  
32.53.720.550

Japan  
0120.8555.90

Asia Pacific  
65.6861.0633

Latin America/Caribbean  
0800.771.7157



For country contact information, visit [bdbiosciences.com/contact](http://bdbiosciences.com/contact)

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for a patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.  
For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

©2020 BD. All rights reserved. Unless otherwise noted, BD, the BD Logo and all other trademarks are the property of Becton, Dickinson and Company or its affiliates.