

## Technical Data Sheet

## Purified Rat Anti- Mouse CD8a

## Product Information

|                  |  |
|------------------|--|
| Material Number: | 553027   |
| Alternate Name:  | Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B            |
| Size:            | 0.5 mg   |
| Concentration:   | 0.5 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*.

## Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE™ antibody format (Cat.No. 553026) for in vitro and in vivo use.

## Application Notes

## Application

|  |                           |
|--|---------------------------|
| Flow cytometry   | Routinely Tested          |
| Immunohistochemistry-frozen                                | Tested During Development |
| Immunohistochemistry-zinc-fixed                            | Tested During Development |
| Immunoprecipitation  | Reported                  |
| Depletion  | Reported                  |
| Blocking   | Reported                  |
| Functional assay   | Reported                  |
| Immunohistochemistry-formalin (antigen retrieval required) | Not Recommended           |

## Recommended Assay Procedure:

This antibody is routinely tested by flow cytometric analysis. Since applications vary, each investigator must determine dilutions appropriate for individual use. For IHC, we recommend use of purified 53-6.7 mAb in our special formulation for immunohistochemistry (Cat. No. 550281).

**IHC of formalin-fixed paraffin-embedded sections is not recommended.** Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

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553027 Rev. 4



## Suggested Companion Products

| Catalog Number | Name   | Size   | Clone  |
|----------------|--|--------|--------|
| 553927         | Purified Rat IgG2a, $\kappa$ Isotype Control | 0.5 mg | R35-95 |
| 550281         | Purified Rat Anti- Mouse CD8a                | 1 mL   | 53-6.7 |
| 553026         | Purified NA/LE Rat Anti-Mouse CD8a           | 0.5 mg | 53-6.7 |
| 554656         | Stain Buffer (FBS)                           | 500 mL | (none) |
| 554657         | Stain Buffer (BSA)                           | 500 mL | (none) |

## 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. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

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