Technical Data Sheet

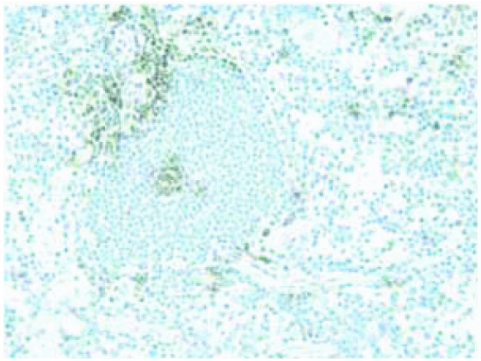
Purified Rat Anti- Mouse CD8a

Product Information

Material Number:	**550281**
Alternate Name:	Cd8a; CD8 alpha chain; Ly-2; Lyt2; Lyt-2; Ly-35; Ly-B
Size:	1 mL
Concentration:	62.5 µg/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, goat serum, 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*.



Immunohistochemical staining of CD8+ T lymphocytes. *Frozen sections of normal mouse spleen were reacted with Purified Rat Anti- Mouse CD8a (Cat. No. 550281), then visualized with Biotin Goat Anti-Rat Ig (Cat. No. 559286), Streptavidin-HRP (Cat. No. 550946), and the DAB detection system (Cat. No. 550880). CD8+ T lymphocytes can be identified by the intense brown labeling of their cell surface membranes.*

Preparation and Storage

Store undiluted at 4°C.

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

Application Notes

Application

Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunohistochemistry-zinc-fixed	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Not Recommended

Recommended Assay Procedure:

Immunohistochemistry: Clone 53-6.7 is recommended to test for immunohistochemical staining of acetone-fixed frozen sections or zinc-fixed

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paraffin sections of mouse spleen or thymus. **IHC of formalin-fixed paraffin embedded sections is not recommended.** The antibody has been reported to stain the membranes of thymocytes and a subpopulation of mature T lymphocytes that are MHC class I restricted. The isotype control recommended for use with this antibody is purified rat IgG2a (Cat. No. 559073). For optimal indirect immunohistochemical staining, the 53-6.7 antibody should be titrated (1:10 to 1:50 dilution and visualized via a three-step procedure in combination with Biotin Goat Anti-Rat Ig (Cat. No. 559286) as the secondary antibody and Streptavidin-HRP (Cat. No. 550946) together with the DAB detection system (Cat. No. 550880). More conveniently, the anti-rat Ig HRP detection kit (Cat. No. 551013) can be used which contains the biotinylated secondary antibody, the antibody diluent, streptavidin-HRP and a DAB substrate for use in the staining procedure.

Suggested Companion Products

Catalog Number	Name	Size	Clone
559073	Purified Rat IgG2a κ Isotype Control	0.25 mg	R35-95
559286	Biotin Goat Anti-Rat Ig	0.5 mg	Polyclonal
550946	Streptavidin HRP	50 mL	(none)
550880	DAB Substrate Kit	500 Tests	(none)
551013	Anti-Rat Ig HRP Detection Kit	200 Tests	(none)
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. 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.
5. This antibody has been developed for the immunohistochemistry application. However, a routine immunohistochemistry test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
6. 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 (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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