# **Technical Data Sheet**

# Purified NA/LE Rat Anti-Mouse CD8a

#### **Product Information**

 Material Number:
 553026

 Alternate Name:
 Ly-2, Lyt-2

 Size:
 0.5 mg

 Concentration:
 1.0 mg/ml

 Clone:
 53-6.7

Storage Buffer: No azide/low endotoxin: Aqueous buffered solution containing no preservative,

 $0.2\mu m$  sterile filtered. Endotoxin level is  $\leq 0.01$  EU/ $\mu g$  ( $\leq 0.001$  ng/ $\mu g$ ) of

protein as determined by the LAL assay.

## Description

The 53-6.7 antibody has been reported to react with 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 (the  $\alpha'$  chain is absent). Subsets of  $\gamma\delta$  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  $\alpha$  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-reaact with CD8  $\alpha$ - and  $\alpha'$ -like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad,  $\alpha$ - and  $\alpha'$ -like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad,  $\alpha$ - and  $\alpha'$ -like polypeptides on subsets of thymic and peripheral lymphocytes in the Egyptian toad

#### **Preparation and Storage**

Store undiluted at 4°C.

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

This preparation contains no preservatives, thus it should be handled under aseptic conditions.

#### **Application Notes**

#### Application

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### **Suggested Companion Products**

| Catalog Number | Name Name                                  | Size   | Clone  |  |
|----------------|--|--------|--------|--|
| 553926         | Purified NA/LE Rat IgG2a κ Isotype Control | 0.5 mg | R35-95 |  |
| 553027         | Purified Rat Anti- Mouse CD8a              | 0.5 mg | 53-6.7 |  |
| 550281         | Purified Rat Anti- Mouse CD8a              | 1.0 ml | 53-6.7 |  |

## **Product Notices**

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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#### References

Alexander-Miller MA, Leggatt GR, Sarin A, Berzofsky JA. Role of antigen, CD8, and cytotoxic T lymphocyte (CTL) avidity in high dose antigen induction of apoptosis of effector CTL. J Exp Med. 1996; 184(2):485-492. (Biology)

Anel A, O'Rourke AM, Kleinfeld AM, Mescher MF. T cell receptor and CD8-dependent tyrosine phosphorylation events in cytotoxic T lymphocytes: activation of p56lck by CD8 binding to class I protein. Eur J Immunol. 1996; 26(10):2310-2319. (Biology: Functional assay)

Bierer BÉ, Sleckman BP, Ratnofsky SE, Burakoff SJ. The biologic roles of CD2, CD4, and CD8 in T-cell activation. *Annu Rev Immunol.* 1989; 7:579-599. (Biology) 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. (Biology)

Hathcock KS. T cell depletion by cytotoxic elimination. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, ed. *Current Protocols in Immunology*. New York: John Wiley and Sons; 1991:3.4.1-3.4.3. (Biology: Depletion)

Janeway CA Jr. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. *Annu Rev Immunol.* 1992; 10:645-674. (Biology)

Ledbetter JA, Herzenberg LA. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol Rev.* 1979; 47:63-90. (Biology: Immunoprecipitation)

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. (Biology: Immunohistochemistry)

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. (Biology: Blocking, Cytotoxicity, Immunoprecipitation)

LeFrancois L. Extrathymic differentiation of intraepithelial lymphocytes: generation of a separate and unequal T-cell repertoire. *Immunol Today.* 1991; 12(12):436-438. (Biology)

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. (Biology)

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)

Mitnacht R, Bischof A, Torres-Nagel N, Hunig T. Opposite CD4/CD8 lineage decisions of CD4+8+ mouse and rat thymocytes to equivalent triggering signals: correlation with thymic expression of a truncated CD8 alpha chain in mice but not rats. *J Immunol.* 1998: 160(2):700-707. (Biology)

Murosaki S, Yoshikai Y, Ishida A, et al. Failure of T cell receptor V beta negative selection in murine intestinal intra-epithelial lymphocytes. *Int Immunol.* 1991; 3(10):1005-1013. (Biology)

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)

Negm HI, Mansour MH, Saad AH, Abdel Halim RS. Structural characterization of an Lyt-2/3 homolog expressed on Bufo regularis lymphocytes. Comp Biochem Physiol B Biochem Mol Biol. 1996; 113(1):79-87. (Biology)

O'Rourke AM, Mescher MF. The roles of CD8 in cytotoxic T lymphocyte function. Immunol Today. 1993; 14(4):183-188. (Biology)

Rocha B, Vassalli P, Guy-Grand D. The extrathymic T-cell development pathway. Immunol Today. 1992; 14(3):140-141. (Biology)

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. (Biology)

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. (Biology: Functional assay)

Traver D, Akashi K, Manz M, et al. Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. *Science*. 2000; 290(5499):2152-2154. (Biology)

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. (Biology: Immunohistochemistry)

Walker ID, Murray BJ, Hogarth PM, Kelso A, McKenzie IF. Comparison of thymic and peripheral T cell Ly-2/3 antigens. *Eur J Immunol.* 1984; 14(10):906-910. (Biology)

Wang J, Klein JR. Thymus-neuroendocrine interactions in extrathymic T cell development. Science. 1994; 265(5180):1860-1862. (Biology)

Zamoyska R. The CD8 coreceptor revisited: one chain good, two chains better. Immunity. 1994; 1(4):243-246. (Biology)

Zamoyska R, Derham P, Gorman SD, et al. Inability of CD8 alpha' polypeptides to associate with p56lck correlates with impaired function in vitro and lack of expression in vivo. *Nature*. 1989; 342(6247):278-281. (Biology: Immunoprecipitation)

Zamoyska R, Vollmer AC, Sizer KC, Liaw CW, Parnes JR. Two Lyt-2 polypeptides arise from a single gene by alternative splicing patterns of mRNA. *Cell.* 1985; 43(1):153-163. (Biology)

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