

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated rat monoclonal anti-mouse CD8 α : Supplied as 25 μ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 53-6.7

Isotype: rat IgG_{2A}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

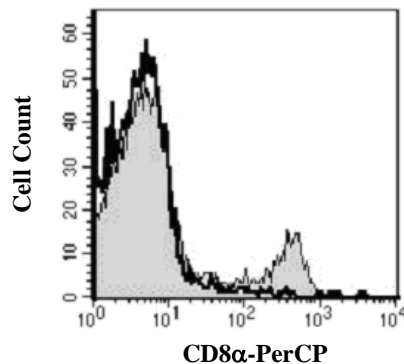
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD8 α within a population and qualitatively determine the density of CD8 α on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with mouse thymus or spleen (Ledbetter, J.A. & L.A. Herzenberg (1979) *Immunol. Rev.* **47**:63 - 90). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Cell surface expression of CD8 α is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



Mouse splenocytes were stained with PerCP-conjugated anti-mouse CD8 α (Catalog # FAB116C, filled histogram) or isotype control (Catalog # IC006C, open histogram).

Background Information

The 53-6.7 monoclonal antibody has been shown to react with both the α and α' chains of mouse CD8 (Ly-2 or Lyt-2), an antigen co-receptor on the T cell surface which interacts with MHC class-I molecules on antigen presenting cells.¹ The CD8 α and CD8 α' chains can form heterodimers by associating with a β -chain which is expressed on a subpopulation of mature T cells.^{2,3} CD8 α expression, in the absence of CD8 β , has been detected on subsets of $\gamma\delta$ TCR-bearing T cells,⁴ on intestinal intrathymic lymphocytes,^{5,6} and dendritic cells.^{7,8}

References

- Bierer, B.E. *et al.* (1989) *Annu. Rev. Immunol.* **7**:579.
- Ledbetter, J.A. *et al.* (1980) *J. Exp. Med.* **152**:280.
- Hayakawa, K. *et al.* (1994) *Science* **263**:1131.
- MacDonald, H.R. *et al.* (1990) *Eur. J. Immunol.* **20**:927.
- Rocha, B. *et al.* (1992) *Immunol. Today* **13**:449.
- Wang, J. & J.R. Klein (1994) *Science* **265**:1860.
- Vermec, D. *et al.* (1992) *J. Exp. Med.* **176**:47.
- Suss, G. & K. Shortman (1996) *J. Exp. Med.* **183**:1789.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

- Cells may be Fc-blocked with 1 μ g of mouse IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 μ L of conjugated antibody was added to 1 - 2.5 x 10⁵ cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
- The cells were resuspended in Flow Cytometry Staining Buffer for analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with PerCP-labeled rat IgG_{2A} antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.