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Monoclonal Antibody to CD8 - PE

Alternate names: CD8 alpha chain, CD8A, MAL, T-cell surface glycoprotein CD8 alpha chain, T-lymphocyte differentiation antigen T8/Leu-2

Catalog No.: CL007R

Quantity: 50 µg

Concentration: 0.1 mg/ml

Background: The CD8 antigen is a cell surface glycoprotein found on most cytotoxic T lymphocytes that mediates efficient cell to cell interactions within the immune system. The CD8 antigen, acting as a coreceptor, and the T cell receptor on the T lymphocyte recognize antigen displayed by an antigen presenting cell (APC) in the context of class I MHC molecules. The functional coreceptor is either a homodimer composed of two alpha chains, or a heterodimer composed of one alpha and one beta chain. Both alpha and beta chains share significant homology to immunoglobulin variable light chains.

Uniprot ID: P01731

NCBI: NP_001074579.1

GeneID: 12525

Host / Isotype: Rat / IgG2b

Clone: YTS169.4

Immunogen: Mouse Ly-2 thymocytes.
Donor: (LOU X DA) F1 rat.
Fusion Partner: Y3/Ag1.2.3.

Format: **State:** Liquid purified IgG fraction
Buffer System: PBS containing 0.02% Sodium Azide and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml
Label: PE

Applications: **Flow Cytometry Analysis** (see Protocols).
This antibody has been reported to be suitable for use in Immunohistochemistry on PFA-Fixed Paraffin-Embedded tissue sections (antigen retrieval is required). (7)
This clone has been reported to work in Immunohistochemistry on Frozen Sections. (6)
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This anti-CD8a (Ly 2) monoclonal antibody reacts with a protein of approximately 30 kDa found on mouse thymocytes and mouse cytotoxic/suppressor T cells. It does not bind to mouse helper/inducer T cells. It binds to T lymphocytes from all mouse strains regardless of phenotypic expression (ie. reacts with T lymphocytes from mouse strains expressing the Ly 2.1 or Ly 2.2 phenotype.). It can be used to investigate the role of T cells in models for infectious disease, autoimmunity, transplantation tolerance and fundamental aspects of

immunology. (1)
It can also be useful to identify/eliminate cytotoxic or suppressor T lymphocytes in vivo or in vitro.
Strains Tested: C57BL/6, BALB/c, AKR/J, C3H/He
Positive: : C57BL/6, BALB/c, AKR/J, C3H/He
Negative: None.

Species Reactivity: **Tested:** Mouse.

Storage: Store the antibody undiluted at 2-8°C.
DO NOT FREEZE!
This product is photosensitive and should be protected from light.
Shelf life: one year from despatch.

General References:
1. Cobbald S.P et al. (1984) Nature. Therapy with monoclonal antibodies by elimination of T cell subsets in vivo 312, 5994, 548-551.
2. Cobbald S.P. et al. 8th International Conference on Lymphatic Tissues and Germinal Centres. Plenum Press (Ed. Klaus G.) in press (1984) Immunosuppression with monoclonal antibodies - rules for effective serotherapy.
3. Aqel N.M. et al. (1984) J. of Immunol. Methods. 69: 207-214. Immunohistological Screening in the selection of monoclonal antibodies: the use of isotype specific antiglobulins.
4. Ledbetter J.A. and Hertzenberg L.A. (1979) Nature. 277: 131-133. Rat x Rat hybrid myelomas and a monoclonal anti-Fd portion of mouse Ig.
5. Mueller, R. et al. (1997) J. of Immunol. 159: 1599-1603. IL-4 Expression by Grafts from Transgenic Mice Fails to Prevent Allograft Rejection.
6. Stevenson, P.G. et al. (1997) J. of Immunol. 159: 1876-1884. Virus Dissemination Through the Brain Parenchyma Without Immunologic Control.
7. Please contact Acris technical services department for further details.

Protocols:

FLOW CYTOMETRY ANALYSIS:

Method:
1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population.
2. Wash 2 times.
3. Resuspend cells to a concentration of 2x10e7 cells/ml Media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test.
4. To each tube add 2.0 µg of this Ab per 10e6 cells.
5. Vortex to ensure through mixing of antibody and cells.
6. Incubate tubes at 4°C for 30 minutes. (It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C in Media B.
8. Resuspend the cell pellet in 50 µl ice cold Media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in phosphate buffered saline. (This stains dead cells by intercalating DNA).

Media:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 ul of 2 M sodium azide in 100 mls.)
B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 ul of 2 M sodium azide in 100 mls.)

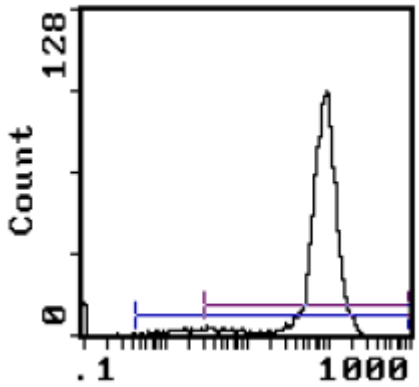
Results - Tissue Distribution

Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 2.0 µg/10e6 cells
Isotypic Control: PE Rat IgG2b,k

Percentage of cells stained above control:
Thymus 66.2%
Spleen 19.6 %
Lymph node 14.0%

Results - Strain Distribution
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.2 µg/10e6 cells
Strains Tested: BALB/c, C57BL/6
Positive: BALB/c, C57BL/6
Negative: none

Pictures:



Cell Source: Thymus. Percentage of cells stained above control: 66.2%