

Monoclonal Antibody to CD8 - FITC

Alternate names: CD8 alpha chain, CD8A, MAL, T-cell surface glycoprotein CD8 alpha chain, T-lymphocyte

differentiation antigen T8/Leu-2

Catalog No.: CL007F

Quantity: 0.1 mg

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: <u>12525</u>

Host / Isotype: Rat / IgG2b Clone: YTS169.4

Immunogen: Murine thymocytes

Format: State: Liquid purified IgG

Purification: Protein G Chromatography

Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total

protein concentration to 4-5 mg/ml.

Label: FITC

Applications: Flow Cytometry.

Immunohistochemistry on frozen sections.

Other applications not tested. Optimal dilutions are dependent on conditions and should

be determined by the user.

Specificity: Antibody CL007 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 (i.e. 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.

Species: Mouse.

Other species not tested.



CL007F: Monoclonal Antibody to CD8 - FITC

Add. Information:

Strain Distribution by Flow Cytometry Analysis:

Procedure: see below

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 0.1 µg/10e6 cells

Strains Tested: BALB/c, C57BL/6 Positive: BALB/c, C57BL/6

Negative: non

Storage:

Store the antibody at 2 - 8 °C up to one month or (in aliquots) at -20 °C for longer. Avoid

repeated freezing and thawing. 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. Agel 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.

Protocols:

FLOW CYTOMETRY ANALYSIS:

Method:

- 1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
- 2. Wash 2 times.
- 3. Resuspend the cells to a concentration of 2x107 cells/ml in media A. Add 50µl of this suspension to each tube (each tube will then contain 1 x 106 cells, representing 1 test).
- 4. To each tube, add 0.1-0.5 μg* of CL007F per 106 cells.
- 5. Vortex the tubes to ensure thorough mixing of antibody and cells.
- 6. Incubate the tubes for 30 minutes at 4°C.

(It is recommended that the tubes are protected from light, since most flurochromes are light sensitive.)

- 7. Wash 2 times at 4°C.
- 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 PBS. This stains dead cells by intercalating in DNA.

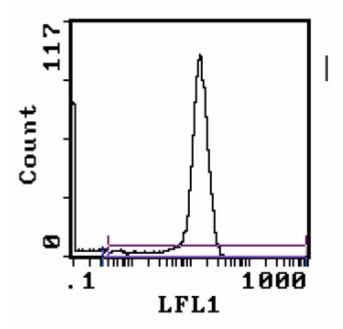
Media:

A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).

B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).



Pictures:



Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 0.1 µg/10e6 cells

Isotypic Control: FITC Rat IgG2b

Cell Source Percentage of cells stained above control: Thymus 74.4%