

# **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.: CL008FX

Quantity: 0.3 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 / IgG2a Clone: CT-CD8a

Immunogen: Murine thymocytes

Format: State: Liquid purifie

**State:** Liquid purified IgG **Purification:** Protein G Chromatography

Buffer System: PBS, 0.09% NaN3 and EIA grade BSA as a stabilizing protein to bring total

protein concentration to 4-5 mg/ml

Label: FITC

**Applications:** Flow Cytometry Analysis (see Protocols).

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

be determined by the user.

Specificity: This anti-mouse CD8a antigen monoclonal antibody recognizes the mouse CD8a chain. The

 $\alpha$  chain of CD8 associates with the CD8 $\beta$  chain to form a CD8 $\alpha/\beta$  heterodimer that is expressed by the majority of thymocytes and by the MHC class I restricted subset of mature T cells1. Mouse CD8 $\alpha$  can also form a CD8 $\alpha/\alpha$  chain homodimer on subsets of CD8 positive cells. For this reason antibodies specific for CD8a rather than CD8b are recommended for a

rigorous delineation of CD8 positive cells.

Species: Mouse.

Other species not tested.



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# **Storage:**

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

Avoid repeated freezing and thawing.

This product is photosensitive and should be proteced from light.

Shelf life: one year from despatch.

General References: 1. Tomonari, k. and Spencer, S. 1990 Epitope-specific binding of CD8 regulates activation of T cells and induction of cytotoxicity. International Immunology 2(12): 1189-1194. 2. Sharon, M., et al. 1999. Interleukin-12 Gene Transfer Results in CD8- Dependant

Regression of Mouse CT26 Liver Tumors. Animals of Surgical Oncology 6(2): 186-194.

### **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 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
- 4. To each tube, add ~1.0 μg\* of this Ab per 1x10e6 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 fluorochromes 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).

# Results:

Mouse Strain: C57BL/6

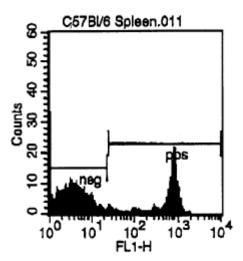
Cell Concentration: 1x10e6 cells per test

Antibody Concentration Used: 1.0 µg/10e6 cells

Isotypic Control: FITC Rat IgG2a



**Pictures:** 



Representative Histogram - Cell Source: CD3e Positive Spleen Cells Percentage of cells stained above control: 34.7%