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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.: CL008F

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: 12525

Host / Isotype: Rat / IgG2a

Clone: CT-CD8a

Immunogen: Murine thymocytes

Format:
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 CD8α chain. The α chain of CD8 associates with the CD8β chain to form a CD8α/β heterodimer that is expressed by the majority of thymocytes and by the MHC class I restricted subset of mature T cells1. Mouse CD8α can also form a CD8α/α 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.

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:

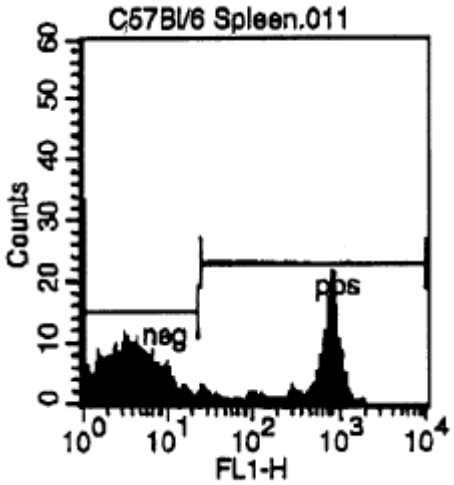
FLOW CYTOMETRY ANALYSIS:

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%