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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

FITC Anti-Rat CD8b Monoclonal Antibody

CL091F
CL091F-5
LOT: 9131

DESCRIPTION:

Cedarlane's anti-rat CD8b monoclonal antibody reacts with the beta chain of the CD8 differentiation antigen. CD8b is expressed on most thymocytes and mature T cytotoxic/suppressor cells (MHC class I restricted). While the CD8a and CD8b form a heterodimer on the surface of thymocytes and thymus-dependent T cytotoxic/suppressor cells, the majority of NK cells, many CD8 T cells from athymic rats, many activated CD4 T cells, and intestinal epithelium lymphocytes (IEL) express CD8a without CD8b. This suggests that expression of the CD8 heterodimer (a/b) is more dependant on intrathymic T cell maturation than that of the homodimer (a/a). The thymus dependence of CD8a/b T cells may be due to a requirement for thymic selection on self MHC class I antigens.

Reported applications for this antibody include flow cytometry, immunoprecipitation and Western blotting. The 3.4.1 antibody also blocks both activation in an allogenic response and cell mediated cytotoxicity by CD8 T cells.

PRESENTATION:

100µg (CL091F) or 500 µg (CL091F-5) FITC conjugated Ig buffered in PBS , 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Store at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles. Avoid prolonged exposure to light.

For more information or to place an order please contact...

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SPECIFICATIONS:

Clone: 3.4.1

Hybridoma Production:

Immunization:

Immunogen: Rat/mouse T cell hybrids expressing CD8
Donor: BALB/c mouse spleen cells

Fusion Partner: X63 Ag.653 myeloma cell line

Specificity: Rat CD8b

Ig Class: mouse IgG_{1,k}

Format: : FITC conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from ascitic fluid via Protein G Chromatography).

Antibody Concentration: 0.1 mg/ml

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 (CL5030).
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.1-0.5 μ g* of **CL091F** or **CL091F-5** per 10^6 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 fluorochemicals are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 μ l ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l 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 μ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μ l of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

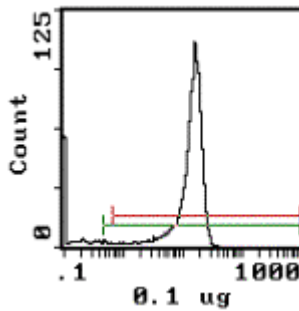
Mouse Strain: Wister

Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: $0.5 \mu\text{g}/10^6$ cells

Isotypic Control: FITC Mouse IgG1

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	90.0%
Spleen Cells	15.2%
Lymph Nodes	17.0%



Cell Source: Thymus

Percentage of cells stained above control: 90.0 %

N.B. Appropriate control samples should always be included in any labeling studies.

*** For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

REFERENCES:

1. Elflein, K., Rodriguez-Palmero, M., Kerkau, T., and T. Hunig. (2003) Immunobiology. 102, 1764 -1770. Rapid recovery from T lymphopenia by CD28 superagonist therapy.
2. Torres-Nagel, N., Kraus, E., Brown, M.H., Tiefenthaler, G., Mitnacht, R., Williams, A.F., and T. Hunig. (1992) Eur. J. Immunol. 22, 2841-2848. Differential thymus dependence of rat CD8 isoform expression*.

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