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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

FITC-Anti-Mouse CD8a (Ly 2.2) Monoclonal Antibody

CL8922F
LOT: 2231

DESCRIPTION:

Cedarlane's anti-mouse CD8a (Ly 2.2) monoclonal antibody reacts with a subpopulation of T-lymphocytes from mouse strains expressing the Ly-2.2 phenotype but does not react with lymphocytes from strains expressing the Ly-2.1 phenotype.
This clone has been shown to work in both cytotoxicity assays and flow cytometry.

PRESENTATION:

100 µg (CL8922F) or 300 µg (CL8922F-3) FITC conjugated Ig buffered in PBS, 0.02% NaN_3 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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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

SPECIFICATIONS:

Clone: AD4(15)

Hybridoma Production:

Immunization: Immunogen: C57BL/6
Donor: B6-Ly-2a

Fusion Partner: Myeloma line P3/X63Ag8

Specificity: Mouse CD8a (Ly2.2)

Ig Class: Mouse IgM

Format: FITC conjugated Ig buffered in PBS, 0.02% NaN_3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from acites via euglobin).

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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 2.0-1.0 µg***** of **CL8922F or CL8922F-3** 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 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:

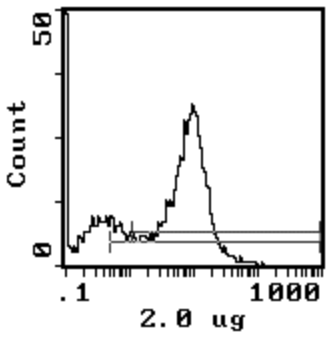
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c
Cell Concentration : $1x10^6$ cells per tests
Antibody Concentration Used: 2.0 µg/10^6 cells
Isotypic Control: FITC Mouse IgM

Cell Source	Percentage of cells stained above control:
Spleen	10.2%
Thymus	66.0%



Cell Source: Thymus
Percentage of cells stained above control: 66.0%

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. Gottlieb, P.D, Marshak-Rothstein, A., Auditore-Hargreaves, K., Berkoben, D.B., August, D.A., and Rosche, R.M. Construction and properties of New Lyt-Congenic Strains and Anti-Lyt-2.2 and Anti-Lyt-3.1 Monoclonal Antibodies. Immunogenetics 10:545 1980.
2. Raulet, D.H., Gottlieb, P.. and Bevan, M.J. Fractionation of Lymphocyte Populations with Monoclonal antibodies Specific for Lyt-2.2 and Lyt-3.1. J. Immunol. 125:1136-1142, 1980.
3. Reilly, E.B., Auditore-Hargreaves, K., Hammerling, U., and Gottlieb, P.D. Lyt-2 and Lyt-3 Alloantigens: Precipitation with Monoclonal and Conventional Antibodies and Analysis on one and two-dimensional Polyacrylamide Gels. J. Immunol. 125:2245-2251, 1980.

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