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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

PE Anti-Rat CD8b Monoclonal Antibody

CL091PE
CL091PE-4
LOT: 9151

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:

50 µg (CL091PE) or 200µg (CL091PE-4) R-PE 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. **DO NOT FREEZE**. Avoid prolonged exposure to light.

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

CEDARLANE® LABORATORIES LIMITED

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in North America

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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: R-PE 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 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®-Rat cell separation medium (CL5040).
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 1.0 µg* of **CL091PE or CL091PE-4** 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.

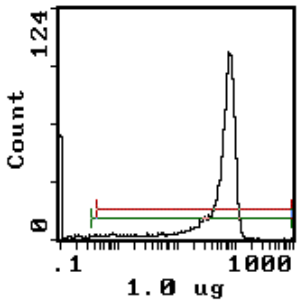
Media:

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:

Rat Strain: Wistar
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used:1.0 µg/10^6 cells
Isotypic Control: PE Mouse IgG_1, k (CLCMG104)
* (T cells isolated with CL102 Cedarlane's Rat T Cell Recovery Column Kit)

Cell Source	Percentage of cells stained above control:
Thymus	92.2%
Spleen	14.6%
Lymph Node	40.3%



Cell Source: Thymus
Percentage of cells stained above control: 92.2%

N.B. Appropriate control samples should always be included in any labelling studies.
***For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

R-Phycoerythrin conjugates are produced under license and protected under Stanford University held patents 4,520,110; 4,542,104; 4,859,582; 5,055,556 (U.S.): 76695 (EPC): 548440 (Australia): 1,179,942 (Canada): and 1,594,827 (Japan).

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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*.

FOR RESEARCH USE ONLY

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