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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Rat CD8b Monoclonal Antibody

CL091AP
CL091AP-2
LOT: 9121

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:

250µg (CL091AP) or 500µg (CL091AP-2) Purified Ig buffered in PBS and 0.02% NaN_3.

STORAGE/STABILITY:

Stable at 4°C. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze thaw cycles.

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

CEDARLANE® LABORATORIES LIMITED

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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 $IgG_{1,K}$

Presentation: Purified Ig buffered in PBS with the addition of 0.02% NaN_3
(Purified from ascites fluid via Protein G chromatography).

FLOW CYTOMETRIY ANALYSIS:

1. Prepare 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 to $1x10^6$ cells in approximately 50 µl Media A in a microcentrifuge tube (i.e. 50 µl of cells resuspended to $2x10^7$ cells/ml; the contents of 1 tube represent 1 test).
4. To each tube add a 0.5 µg * of **CL091AP or CL091AP-2.**
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody **CLCC30204** (PE Goat anti-mouse IgG at 1:500 dilution.
9. Incubate tubes at 4°C for 30-60 minutes.
   (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in Media B.
11. Resuspend the cell pellet in 50 µl ice cold Media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in phosphate buffered saline. (This stains dead cells by intercalating DNA).

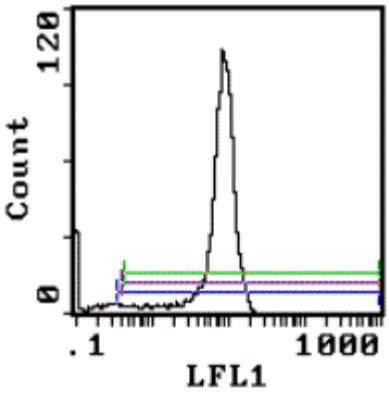
MEDIA:

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

FLOW CYTOMETRIC ANALYSIS

Rat Strain: Wistar
Cell Concentration: $1x10^6$ cells per test
Antibody Concentration: 0.5 µg/10^6 cells
Isotypic Control: Purified Mouse IgG_1

CELL SOURCE	PERCENT STAINING
Thymus	83.36%
Spleen	15.5%
Lymph Node	17.4%



Cell Source: Thymocytes
Percentage of Cells Stained Above Control: 83.3%

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.**

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*.

FOR RESEARCH USE ONLY

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