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TECHNICALLY Speaking

**Place your order with CEDARLANE® or your local distributor.**

*Please contact CEDARLANE® for lot specific information.*

# Biotin Anti-Rat CD8b Monoclonal Antibody

**CL091B**
**CL091B-5**
**LOT: 9141**

**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 (CL091B) or 500 µg (CL091B-5) FITC conjugated Ig buffered in PBS , 0.02% $NaN_3$ and EIA grade BSA as a stabalizing 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.

*Continued Overleaf…*

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: : Biotin 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 $1x10^6$ cells, representing 1 test).
4. To each tube, add 50µl of a 1.0 g* dilution of **CL091B** or **CL091B-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.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody **CLCSA1004** (PE Streptavidin) at 1:500 dilution.
9. Incubate the 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.
Transfer to suitable tubes for flow cytometric analysi 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).

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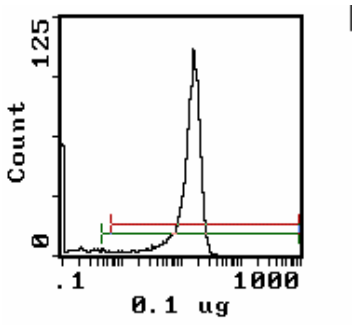
Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: Wister
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 0.5 µg/$10^6$ cells
Isotypic Control: Biotin Mouse IgG1

| Cell Source | Percentage of cells stained above control: |
|---|---|
| Thymus | 90.0% |
| Spleen Cells | 15.2% |
| Lymph Nodes | 17.0% |




Cell Source: Thymus
Percentage of cells stained above control: 90.0 %
Representative Histogram

**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**: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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*.


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