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TECHNICALLY *Speaking*

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Please contact CEDARLANE® for lot specific information.

Biotin Rat Anti-Mouse Ly-6A/E Monoclonal Antibody

CL8948B

CL8948B-3

LOT:

DESCRIPTION:

This monoclonal antibody reacts with Ly-6A.2 and Ly-6E.1, members of the Ly-6 multigene family. Ly-6A/E molecules are expressed on multipotent hematopoietic stem cells¹ activated T cells, and peripheral B cells on mice that express the Ly-6.2 haplotype.

This antibody can be used in immunostaining for flow cytometry^{1,4,5}, Western Blot analysis³, and immunoprecipitation^{1,2}. This clone is reported to work in immunohistochemistry with frozen tissue sections¹.

PRESENTATION:

100 µg (CL8948B) or 300 µg (CL8948B-3) Biotin conjugated Ig buffered in PBS, containing 0.1% sodium azide (NaN₃) as a preservative. A highly purified grade of BSA has been added as a stabilizing protein to bring the total protein concentration to 4-5 mg/ml after conjugation.

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. If the reagent is being diluted, it is recommended that only the quantity to be used within one week be diluted. Check label for expiry date.

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

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SPECIFICATIONS:

Clone: D7

Specificity: Mouse Ly-6A/E

Ig Class: Rat IgG_{2a}

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. Re-suspend 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 $\sim 0.25 \mu\text{g}^*$ of **CL8948B** or **CL8948B-3**.
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 μ l of detection reagent CLCSA1001 (Streptavidin-FITC) at 1:50 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. Re-suspend the cell pellet in 50 μ l ice cold media B.
12. 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:

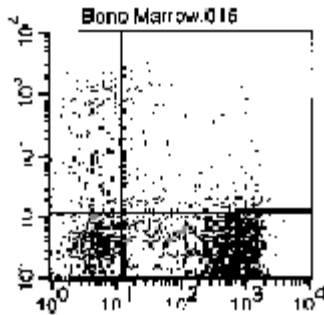
(Representative Dot Plot)

Mouse Strain: C57BL/6

Cell Concentration: 1×10^6 cells per test

Antibody Concentration used: $\sim 0.25 / 10^6$ cells

Isotypic Control: Biotin Rat IgG2a (CLCR2A15)



Cell Source: mouse bone marrow cells

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.**

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1. Ortega, G., P.E. Korty, E.M. Sherach, and T.R. Malek. 1986. Role of Ly-6 in lymphocyte activation. I. Characterization of a monoclonal antibody to a nonpolymorphic Ly-6 specificity. *J. Immunol.* **137**: 3240-3246.
2. Palfree, R.G.E., F.J. Dumont, and U. Hammerling. 1986. Ly-6A.2 and Ly-6E.1 molecules are antithetical and identical to MALA-1. *Immunogenetics* **23**: 197-207.
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