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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Biotin Anti-Mouse I-A^b Monoclonal Antibody

**CL8702B
CL8702B-3
LOT: 8241**

DESCRIPTION:

Cedarlane's anti-mouse I-A^b monoclonal antibody reacts with the I-A^b encoded MHC class II antigen expressed on mouse strains of the H-2^b haplotype. It also reacts with the I-A^d encoded MHC class II antigen expressed on mouse strains of the H-2^d haplotype. Class II antigens are most highly expressed on antigen-presenting cells including B cells, macrophages, dendritic cells and certain epithelial cells.

This antibody is suitable for use in flow cytometry.

PRESENTATION:

100µg (CL8702B) or 300µg (CL8702B-3) Biotin conjugated Ig buffered in PBS, 0.02% NaN₃ 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.

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: 28-16-8S

Specificity: Mouse MHC class II I-A^b, cross-react with I-A^d

Ig Class: Mouse IgM

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. Resuspend 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 0.2 μ g – 0.5 μ g* of **CL8702B** or **CL8702B-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.
7. Wash 2 times at 4°C.
8. Add 100 μ l of secondary antibody **CLCSA1001** (Streptavidin-FITC) at a 1/500 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
11. Resuspend 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).

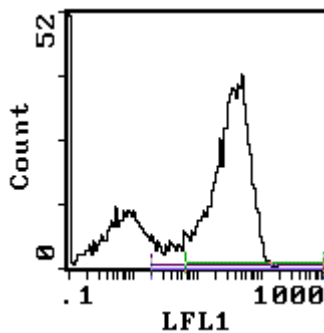
Results:Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: 0.5 μ g/ 10^6 cells

Isotypic Control: Biotin Mouse IgM (CLCMGM15)



Cell Source: Spleen

Percentage of cells stained above control: 64.8%

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. Ozato, K. and Sachs, D. H. 1981 Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2b haplotype reveal genetic control of isotype expression. J. Immunol. 126:317-322.

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