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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^d Monoclonal Antibody

**CL8713B
LOT: 1341**

DESCRIPTION:

Cedarlane's CL8713B is a cytotoxic monoclonal antibody specific for cells expressing the Ia antigen coded for by the A subregion of the d, b, p, and q haplotypes. (ie. I-A^{d,b,p,q})

PRESENTATION:

100 µg 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: 34-5-3s

Hybridoma Production:

Immunization: Immunogen: BDF spleen

Donor: C3H/He spleen

Fusion Partner: SP2/0-Ag14

Specificity: Mouse I-A^d

Ig Class: Mouse IgG_{2a}

Format: 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. (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[®]-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 0.2-0.5 μ g* of **CL8713B** 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 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:

Mouse Strain: BALB/c

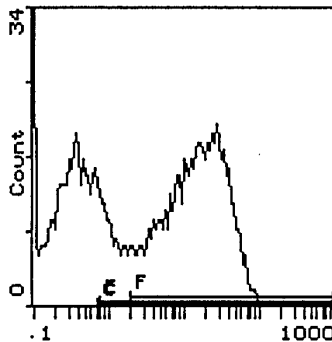
Cell Concentration : 1×10^6 cells per tests

Antibody Concentration Used: $0.5 \mu\text{g}/10^6$ cells

Isotypic Control: Biotin Mouse IgG_{2a}

Cell SourcePercentage of cells stained above control:

Spleen	47.0%
Lymph Node	21.3%
Thymus	24.6%



LFL1

Cell Source: Spleen

Percentage of cells stained above control:47.0%

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.**

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2

Antibody Concentration Used: 0.2 µg/10⁶ cells

Strains Tested: A.TH, A.TL, C57BL/6, C3H/He, DBA/1

Positive: C57BL/6, DBA/1

Negative: A.TH, A.TL, C3H/He

REFERENCES:

1. Ozato, K. et al. 1982. Monoclonal Antibodies to Mouse Major Histocompatibility Complex Antigens. *Transplantation*. **34**:113-120.
2. Ahn, H.J. et al. 1997. A Mechanism Underlying Synergy Between IL-12 and IFN-γ-Inducing Factor in Enhanced Production of IFN-γ. *Journal of Immunology*. **159**: 2125-2131.

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