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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^P Monoclonal Antibody

CL8708B
LOT: 3842

DESCRIPTION:

Cedarlane's anti-I-A^P monoclonal antibody is a cytotoxic antibody which defines a public I-A antigen. This antibody reacts with I-A antigen from the following I-A haplotypes: I-A^{p.k.q.r.s.b}. Using recombinant strains, reactivity against the b haplotype has been localized to the A^b subregion. This antibody can be used to quantitate or eliminate I-A bearing cells or for precipitating I-A antigen.

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...

CEDARLANE®
LABORATORIES LIMITED



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or visit our website for a list of our international distributors including contact information
website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

SPECIFICATIONS:

Clone: 7-16.17

Hybridoma Production:

Immunization: Immunogen: B10.P

Donor: BALB/c

Fusion Partner: SP2/0

Specificity: Mouse-I-A^{p,k,q,r,s,b}

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 1.0 - 0.5 μ g* of **CL8708B** 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: BDP

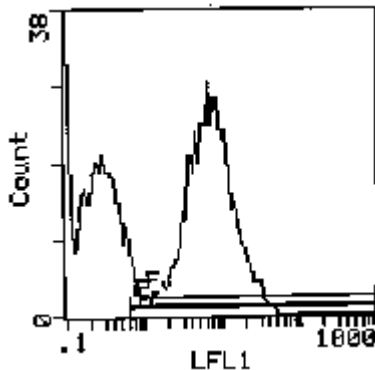
Cell Concentration : 1×10^6 cells per tests

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

Isotypic Control: Biotin Mouse IgG_{2a}

Cell SourcePercentage of cells stained above control:

Thymus	36.5%
Spleen	51.5%
Lymph Node	16.3%
Bone Marrow	24.4%



Cell Source: Spleen

Percentage of cells stained above control: 51.5%

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.**

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2

Antibody Concentration Used: 0.5 µg/10⁶ cells

Strains Tested:

Strain	H-2 Loci Alleles								+/-
	<u>K</u>	<u>A_β</u>	<u>A_α</u>	<u>E_β</u>	<u>E_α</u>	<u>C4</u>	<u>C4S</u>	<u>D</u>	
BDP	s	s	s	s	s	s	s	d	+
A.TH	s	s	s	s	s	s	s	d	+
C3H/He	k	k	k	k	k	k	k	k	+
C57BL/6	b	b	b	b	b	b	b	b	+
BALB/c	d	d	d	d	d	d	d	d	-

For a more detailed strain distribution - see reference 1.

REFERENCES:

1. Harmon, R.C., Stein, N., Frelinger, J.A., 1983. Immunogenetics 18:541-545.

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