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# TECHNICALLY *Speaking*

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## **Biotin Anti-Mouse CD11b Monoclonal Antibody**

**CL8941B**

**CL8941B-3**

**LOT:4142**

### **DESCRIPTION:**

Cedarlane's anti-mouse CD11b (Mac-1; Ly 40) monoclonal antibody is specific for the 170 kDa  $\alpha$  subunit of Mac-1 which mediates adhesion to ICAM-1 (CD54) and C3bi. Mac-1 is expressed on granulocytes, macrophages, natural killer cells, and B-1 cells in the peritoneal and pleural cavities. Mac-1 is up-regulated on neutrophils after activation. This particular clone blocks cell adherence and C3bi binding but does not block cell mediated lysis (1,2,3,4,5,6).

Applications include flow cytometry, *in vitro* and *in vivo* blocking, immunohistochemistry for both paraffin embedded and acetone-fixed frozen sections 1-20  $\mu$ g/ml (#7). Also used for immunoprecipitation and western blotting.

### **PRESENTATION:**

100  $\mu$ g (CL8941B) or 300  $\mu$ g (CL8941B-3) Biotin conjugated Ig buffered in PBS, 0.02%  $\text{NaN}_3$  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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**SPECIFICATIONS:**

Clone: M1/70.15

Hybridoma Production:

Immunization: Immunogen: C57BL/10 spleen cell enriched for  
T lymphocytes  
Donor: DA rat spleen

Fusion Partner: NS-1

Specificity: Mouse CD11b (MAC-1, Ly-40)

Ig Class: Rat IgG<sub>2b</sub>

Format: Biotin conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> 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<sup>®</sup>-M cell separation medium (CL5030).
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 0.2-0.05  $\mu$ g\* of **CL8941B or CL8941B-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  $\mu$ 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  $\mu$ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ 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  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

Results:Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

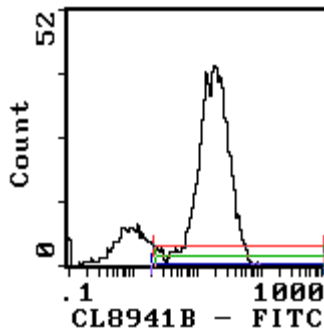
Cell Concentration :  $1 \times 10^6$  cells per test

Antibody Concentration Used: 0.2  $\mu$ g/ $10^6$  cells

Isotypic Control: Biotin Rat IgG<sub>2b</sub>

Cell SourcePercentage of cells stained above control:

Bone Marrow Macrophages	76.1%
Peritoneal Macrophages	77.7%
Thymus	5.2%



Cell Source: Bone Marrow

Percentage of cells stained above control: 76.1%

**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.**

**REFERENCES:**

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