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## Biotin Anti-Mouse CD40 Monoclonal Antibody

**CL8939B**

**CL8939B-3**

**LOT: 0300A0509**

### **DESCRIPTION:**

Cedarlane's anti-mouse monoclonal antibody reacts with CD40 which is expressed on B-lymphocytes and on a subset of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the adult (but not neonatal) spleen<sup>1</sup>. The level of CD40 on B cells is enhanced following activation by LPS<sup>1</sup>. The interactions of CD40 with its ligand CD40L are responsible for generating humoral responses, germinal center formation, and generating B cell memory<sup>2</sup>. Stimulating cells with immobilized 3/23 mAb increases expression of CD86 (B7.2) on B cells<sup>1,3</sup>. Though the 3/23 mAb itself is a weak mitogen, it produces a synergistic proliferative B cell response with IL-4 or with mitogenic antibodies against surface Ig<sup>1,3</sup>. Costimulation with 3/23 mAb and IL-4 rescues B cells from apoptosis induced by hypercross-linking of sIgM or sIgD<sup>3</sup>.

This antibody is suitable for use in flow cytometry. Other applications of this antibody include IHC of acetone-fixed frozen sections<sup>2</sup>.

### **PRESENTATION:**

100 µg (CL8939B) or 300 µg (CL8939B-3) Biotin conjugated Ig buffered in PBS, 0.1% NaN<sub>3</sub> 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. Check label for expiry date.

### **SPECIFICATIONS:**

Clone: 3/23

Specificity: Mouse CD40

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

Antibody Concentration: 0.1 mg/ml

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

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## 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  $\sim 1.0 \mu\text{g}$ \* of **CL8939B** or **CL8939B-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 **CLCSA1004** (Streptavidin-PE) at a 1:50 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes be 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:

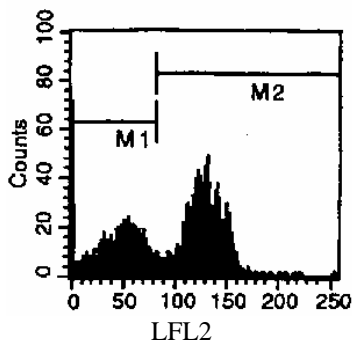
**(representative histogram)**

Mouse Strain: BALB/c

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

Antibody Concentration Used:  $1.0 \mu\text{g}/10^6$  cells

Isotypic Control: Biotin Rat IgG<sub>2a</sub> (**CLCR2A15**)



Cell Source: Spleen

Percentage of cells stained above control: 61.8%

**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.**

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**REFERENCES:**

1. Hasbold, J., C. Johnson-Le`ger, C.J. Atkins, E.A. Clark and G.G.B. Klaus. 1994. Properties of mouse CD40: cellular distribution of CD40 and B cell activation by monoclonal anti-mouse CD40 antibodies. *Eur.J.Immunol.* **24**: 1835-1842
2. Foy, T.M., J.D. Laman, J.A. Ledbetter, A.Aruffo, E.Claassen, and R.J. Noelle. 1994. Gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. *J.Exp.Med.* **180**: 157-163.
3. Parry, S.L., J. Hasbold, M. Holman, and G.G.B. Klaus. 1994. Hypercross-linking surface IgM or IgD receptors on mature B cells induces apoptosis that is reversed by co-stimulation with IL-4 and anti-CD40. *J.Immunol.* **152**: 2821-2829

**Laboratory Reagent For Research Use Only**

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