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**PE Anti-Mouse CD40  
Monoclonal Antibody**

**CL8939PE**  
**CL8939PE-3**  
**LOT: 03020406**

**DESCRIPTION:**

Cedarlane's anti-mouse monoclonal antibody (clone 3/23) 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>.

The 3/23 clone is suitable for use in flow cytometry. This clone is reported to be suitable for use in immunohistochemistry of acetone fixed frozen tissue sections<sup>2</sup>.

**PRESENTATION:**

50 µg (CL8939PE) or 300 µg (CL8939PE-3) R-PE conjugated Ig buffered in PBS, 0.1% sodium azide (NaN<sub>3</sub>) and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

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

**CEDARLANE®**  
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**STORAGE/STABILITY:**

Store at 4°C. **DO NOT FREEZE.** Avoid prolonged exposure to light. If the reagent is being diluted, it is recommended that only the quantity to be used within one week be diluted. Check label for expiry date.

**SPECIFICATIONS:**

Clone: 3/23

Specificity: Mouse CD40

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

Immunogen: mouse CD40-human IgG1 fusion protein<sup>1</sup>

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 preparation 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  $\sim 0.25 \mu\text{g}^*$  of **CL8939PE** or **CL8939PE-3** per  $1 \times 10^6$  cells.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.  
(It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.
9. 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).

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Results:

Tissue Distribution by Flow Cytometry Analysis:  
**(Representative Histogram)**

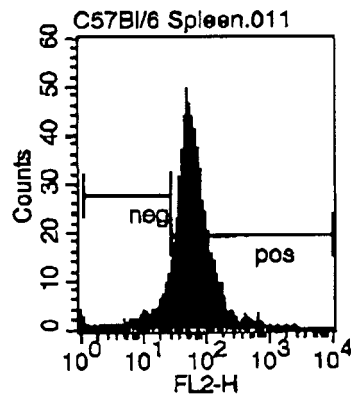
Mouse Strain: C57Bl/6

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

Antibody Concentration Used: 0.25 ug

Isotype Control: PE Rat IgG2a (CLCR2A04)

Cell Source: CD45R (B220)<sup>+</sup> gated spleen cells



Percentage of cells stained above control: 90.7%

**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:**

1. Hasbold, J., C. Johnson-Leger, 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.

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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.

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