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for the Science of Tomorrow™

## Purified Anti-Mouse Macrophage (F4/80) Monoclonal Antibody

**CL8940AP**  
**LOT:**

### **DESCRIPTION:**

Cedarlane's anti-mouse F4/80 monoclonal antibody reacts with the mouse macrophage F4/80 antigen, which is a 160kD plasma membrane component on mouse mononuclear phagocytes. The F4/80 antigen is found on most macrophages, and on macrophage precursors from M-CFC onward. Expression of this antigen is increased upon maturation. F4/80 is found in low levels on activated macrophages and eosinophils. Dendritic leukocytes may be negative or express F4/80 in low levels.

Applications include flow cytometry. This clone is also reported to work in immunohistochemistry, both frozen and paraffin sections (#5).

### **PRESENTATION:**

200 µg purified Ig buffered in PBS and 0.09% NaN<sub>3</sub>

### **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.

### **SPECIFICATIONS:**

Clone: C1:A3-1

Specificity: Mouse Macrophage (F4/80)

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

Format: Purified Ig buffered in PBS and 0.09% NaN<sub>3</sub>.

Antibody Concentration: 1.0mg/ml

*Continued...*

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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 **CL8940AP**.
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 **CLCC40001** (FITC Goat anti-rat IgG (H+L)) at 1:500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes.  
(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
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).

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1. Handbook of Experimental Immunology, Ed. Weir, D.M. (Chapter 43).
2. Szu-Hee Lee, Starky, P.M., Gordon, S. "Quantitative Analysis of Total Macrophage Content in Adult Mouse Tissues", J. Exp. Med. (1985), Volume 161 pp 475-489.
3. Hume, D.A., Perry, V.H., Gordon, S. "The Mononuclear Phagocyte System of the Mouse Defined by Immunohistochemical Localizations of Antigen F4/80: Macrophages Associated with Epithelia", The Anatomical Record (1984), Volume 210, pp 503-512.
4. Eur. J. Immunol. (1981), Volume 11, pp 805-815.
5. Whiteland, J.L et al (1995). Immunohistochemical detection of T cell subsets and other leukocytes in paraffin embedded rat and mouse tissues with monoclonal antibodies .J. Histochem. Cytochem. 43: 313-320.

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