Purified Anti-Mouse MAC-3 
Monoclonal Antibody

CL8943AP
CL8943AP-3
LOT: 4321

DESCRIPTION:
Cedarlane’s anti-mouse MAC-3 monoclonal antibody recognizes the MAC-3 antigen which is found on macrophages and some nonlymphoid tissues, but not on lymphocytes. It is a glycoprotein showing a broad band in SDS-PAGE, and is synthesized by macrophages (average molecular weight 92-110,000 daltons, depending on the origin of macrophages).
It reacts with peritoneal exudate macrophages where the exudate is provoked by thioglycollate, protease peptone, L. monocytogenes, lipopolysaccharide and concanavalin A. It does not react with thymocytes, spleen, lymph node or bone marrow cells in immunofluorescent flow cytometry.
MAC-3 is a general marker for macrophages and can be used to distinguish these cells from lymphocytes. Its Mr 110,000 and cell distribution differentiate MAC-3 from MAC-2 and MAC-1 antigens.

PRESENTATION:
100 µg (CL8943AP) or 300ug (CL8943AP-3) purified Ig buffered in PBS and 0.02% NaN₃.

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: M3/84

Hybridoma Production:
  Immunization: Immuneogen: Immunoadsorbent purified mouse macrophage glycoprotein fraction.
  Donor: Rat spleen
  Fusion Partner: NS-1

Specificity: Mouse MAC-3

Ig Class: Rat IgG1,κ

Antibody Concentration: 1.0 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 2x10^7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10^6 cells, representing 1 test).
4. To each tube, add 1.0 - 0.5 µg* of CL8943AP or CL8943AP-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 µl 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 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl 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 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c
Cell Concentration: 1 x 10⁶ cells per tests
Antibody Concentration Used: 0.5 µg/10⁶ cells
Isotypic Control: Purified Rat IgG₁k

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.
REFERENCES:


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