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Monoclonal Antibody to Neutrophils - FITC

Catalog No.: CL050F

Quantity: 0.1 mg

Concentration: 0.1 mg/ml

Background: Neutrophils constitute the principal cells of acute inflammation.Their entrance is stimulated by chemotactic factors secreted from injured cells, resident tissue macrophages, and complement activation. Neutrophils can also be activated by Fc region of antibodies, and by T-cell derived cytokines. A very potent chemotactic factor for neutrophils is C5a, a peptide product of complement activation. Neutrophils contain abundant cytoplasmic granules, which contain toxic proteins. (clinical correlete:type III, roitt) They are short lived cells. They engulf microorganisms, destroy it, but die quickly thereafter. Neutrophils are activated by antibodies, complement and cytokines.

Host / Isotype: Rat / IgG2a

Clone: 7/4

Immunogen: Cultured bone marrow cells.

Format:
State: Liquid purified Ig fraction.
Buffer System: PBS
Preservatives: 0.09% Sodium Azide
Stabilizers: EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml
Label: FITC

Applications:
Flow Cytometry.
This Clone has been reported to work in **Immunohistochemistry on Frozen and Paraffin Sections.**
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:

This antibody is specific for detecting Mouse Neutrophils.
Strains reported to be Positive for the 7/4 clone are: AKR, C57BL/6, C57BL/10,C58, DBA/2, MF1, NZB, NZW, SJL, Swiss (PO) and 129J.
Strains reported to be Negative/Weak for the 7/4 clone are: A2G, A/Sn, ASW, BALB/c, C3H/HEH and CBA.T6T6.
Actual results of Flow Cytometric analysis
Mouse strain: C57BL/6
Cell Source: Peripheral Blood Leukocytes
Cell Concentration: 1x10e6 cells per test
Antibody concentration: 1.0 µg/10e6 cells
Isotypic Control: FITC Rat IgG2a
Percentage of cells stained above control: 2.46%
Species: Mouse.
Other species not tested.

Storage:

Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
This product is photosensitive and should be protected from light.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References:

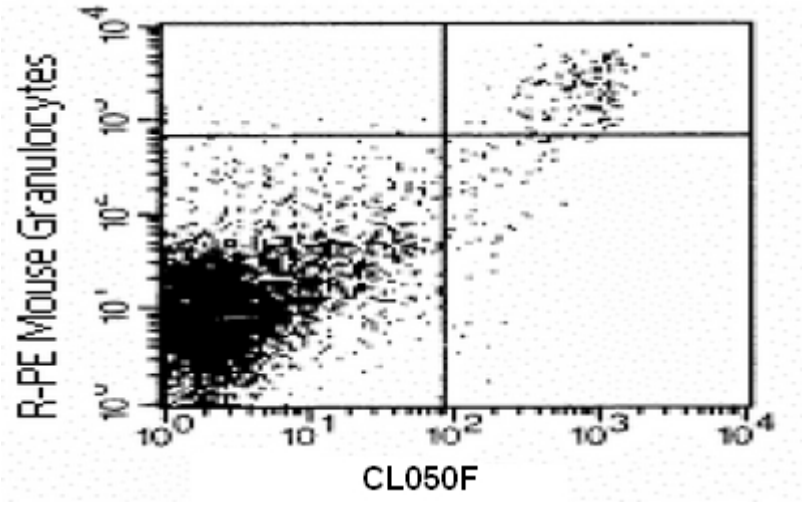
1. Hirch, S. and Gordon, S. (1983). Polymorphic expression of a neutrophil differentiation antigen revealed by a monoclonal antibody 7/4. Immunogenetics 18:229-239.
2. Gordon, S. et al. (1992). Antigen markers of macrophage differentiation in murine tissues. Curr. Top. Microbiol. Immunol. 181: 1-37.

Protocols:

<u>FLOW CYTOMETRY ANALYSIS</u>
Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population using a NH4Cl lysing buffer.
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 $1 x 10^6$ cells, representing 1 test).
4. To each tube, add ~1.0 µg of CL050F 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.
(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 µl ice cold media B.
9. 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).

Pictures:



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