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Monoclonal Antibody to Neutrophils - FITC

Catalog No.: CL050FX

Quantity: 0.3 mg

Concentration: 0.1 mg/ml

Host / Isotype: Rat / IgG2a

Clone: 7/4

Immunogen: Cultured bone marrow cells.

Format:
State: Liquid Ig fraction.
Buffer System: PBS with 0.02% sodium azide as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml
Label: FITC

Applications:
This antibody is suitable for use in Flow cytometry.
This clone has also been reported to be useful for Immunohistochemistry (both 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.

Add. Information:
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: 21.84%

Storage:
Store the antibody at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid prolonged exposure to light.
Avoid freeze/thaw cycles.
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:

FLOW CYTOMETRY ANALYSIS:
Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population using a NH_4Cl lysing buffer.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
4. To each tube, add ~1.0 µg of CL050F per 10e6 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).