

Product datasheet for **CL050P**

Neutrophils Rat Monoclonal Antibody [Clone ID: 7/4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	7/4
Applications:	FC
Recommended Dilution:	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).
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
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.
Formulation:	PBS containing 0.09% Sodium Azide as preservative. State: Purified State: Liquid purified IgG fraction.
Concentration:	lot specific
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.



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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 correlate: 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.

Note: Protocol: **FLOW CYTOMETRY ANALYSIS:**

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 2x10⁷ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10⁶ cells, representing 1 test).
4. To each tube, add ~1.0 µg* of this Ab.
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 (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).

* Appropriate control samples should always be included in any labelling studies