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**Purified Rat Anti-Mouse Ly-6A/E (Sca-1)
Monoclonal Antibody**

CL8948AP
LOT: B152231

DESCRIPTION:

This monoclonal antibody reacts with Ly-6A.2 and Ly-6E.1, members of the Ly-6 multigene family. Ly-6A/E molecules are expressed on multipotent hematopoietic stem cells¹ activated T cells, and peripheral B cells on mice that express the Ly-6.2 haplotype.

This antibody can be used in flow cytometry^{1,4,5}, Western Blot analysis³, immunoprecipitation^{1,2}, and immunohistochemistry with frozen tissue sections¹.

PRESENTATION:

200 µg purified Ig buffered in PBS and 0.09% sodium azide (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: D7

Specificity: Mouse Ly-6A/E (Sca-1)

Ig Class: Rat IgG_{2a}

Antibody Concentration: 0.5 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. Re-suspend 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 ~ 0.25 µg* of **CL8948AP**.

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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. Re-suspend 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).

N.B. Appropriate control samples should always be included in any labelling studies.

***For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

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