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Product datasheet for CL046P

Ly6g Rat Monoclonal Antibody [Clone ID: RB6-8C5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	RB6-8C5
Applications:	CT, FC, IHC, WB
Recommended Dilution:	This antibody is suitable for studies of myeloid differentiation stages and their regulations by cytokines. Applications include Flow Cytometry (1,2,3) complement-mediated depletion (4), Western blot staining (5) and both Frozen and Paraffin Sections (6).
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Specificity:	This monoclonal antibody reacts with the myeloid differentiation antigen Gr-1. (1,2). This 25-30 kDa cell surface antigen is expressed on myeloid cells but not lymphoid or erythroid cells. The expression of the Gr-1 antigen increases with granulocyte maturation (3) as shown by the distinct populations of bone-marrow cells this monoclonal antibody labels: negative, low positive and high positive. Expression is transient on cells of monocytic lineage (3).
Formulation:	PBS State: Purified State: Liquid purified IgG fraction Preservative: 0.02% Sodium Azide
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	Unconjugated
Storage:	Store 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.
Gene Name:	lymphocyte antigen 6 complex, locus G

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Database Link: Entrez Gene 546644 Mouse
P35461

Background: The Gr-1 antigen is primarily a marker of myeloid differentiation. In the bone marrow the level of Gr-1 expression is low on immature myeloblasts and increases as the myeloid cells mature to granulocytes. Gr-1 is also expressed on macrophages and transiently on differentiating monocytes. Expression of Gr-1 on a subpopulation of lymphocytes has also been reported.

Synonyms: Gr-1 Granulocyte marker

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

Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with cell separation medium.
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 0.1-0.2 µg* of CL046P.
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) .
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
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:
Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.1 µg/10e6 cells
Isotypic Control: Rat IgG2b

Cell Source-Percentage of cells stained above control:
Thymus: 2.0%
Whole Blood Monocytes: 79.1%

Bone Marrow Macrophages: 87.5%

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.

Strain Distribution by Flow Cytometry Analysis:
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.1 µg/10e6 cells
Strains Tested: BALB/c, C57BL/6, CBA, C3H/He, AKR
Positive: BALB/c, C57BL/6, CBA, C3H/He, AKR
Negative: none

Product images: