

## Product datasheet for **CL046**

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

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	RB6-8C5
Applications:	CT, FC, WB
Recommended Dilution:	Flow Cytometry. Complement mediated depletion. Western blot. Immunohistochemistry on Frozen and Paraffin Sections.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Specificity:	This antibody 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:	State: Ascites State: Lyophilized Ascites (non-sterile filtered to 0.45 µm).
Reconstitution Method:	Restore with 0.5 ml of cold distilled water.
Conjugation:	Unconjugated
Storage:	Prior to reconstitution and Following reconstitution 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.
Gene Name:	lymphocyte antigen 6 complex, locus G
Database Link:	<a href="#">Entrez Gene 546644 Mouse P35461</a>



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**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 Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 50  $\mu$ l of a 1:5000-1:10000 dilution\* 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  $\mu$ l of secondary antibody (FITC Goat anti-rat IgG (H+L)) at a 1:500 dilution.
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  $\mu$ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l 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  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

**Results - Tissue Distribution:**

Mouse Strain: BALB/c

Cell Concentration:  $1 \times 10^6$  cells per test

Antibody Concentration Used: 50 $\mu$ l of a 1:5000 dilution/ $10^6$  cells

Isotypic Control: Rat IgG2b

**Cell Source Percentage of cells stained above control:**

Thymus: 9.7%

Whole Blood - Granulocytes: 98.6%

Whole Blood - Monocytes: 93.5%

Bone Marrow - Granulocytes: 88.9%

Bone Marrow - Monocytes: 93.2%

**Results - Strain Distribution:**

Cell Concentration:  $1 \times 10^6$  cells per test

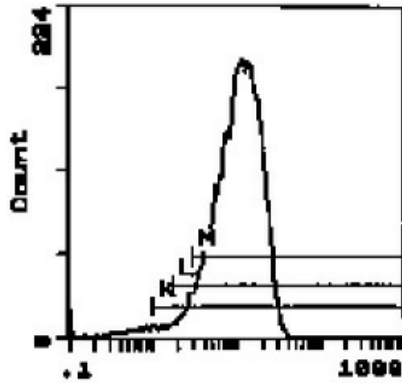
Antibody Concentration Used: 1:5000 in 50 $\mu$ l/ $10^6$  cells

Strains Tested: BALB/c, C57BL/6, CBA, C3H/He, AKR

Positive: BALB/c, C57BL/6, CBA, C3H/He, AKR

Negative: none

**Product images:**



Cell Source: Bone Marrow Monocytes -  
Percentage of cells stained above control: 93.2%