Acris Antibodies GmbH

Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

US office:
Acris Antibodies, Inc.
San Diego, CA
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

Monoclonal Antibody to Ly6G / GR1 Neutrophil Marker - FITC

Alternate names: Gr-1 Granulocyte marker

Catalog No.: CL046FX

Quantity: 0.3 mg

Concentration: 0.1 mg/ml

Background: GR-1 is a 25-30 kDa cell surface antigen and 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).

Uniprot ID: P35461

NCBI: 10090

Host / Isotype: Rat / IgG2b

Clone: RB6-8C5

Format:
State: Liquid Ig fraction
Purification: Protein G chromatography
Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml
Label: FITC
Absorption / Emission: 495 nm / 528 nm

Applications: Flow cytometry (1,2,3).
Western blot (5).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with the myeloid differentiation antigen GR-1 (1,2).
Species: Mouse.
Other species not tested.

Storage: Store the antibody at 2 - 8 °C for up to one month. For long term storage, aliquot and freeze unused portion at -20 °C in volumes appropriate for single usage. Avoid repeated freezing and thawing This product is photosensitive and should be protected from light.
Shelf life: one year from despatch.

General References:
1. Spangrude, G.J., et al.. Purification and characterization of mouse hematopoietic stem cells. Science 241:58-62, 1991.
2. Muller, C. E. et al. Isolation of two early B lymphocyte progenitors from mouse marrow: a committed pre-pre-B cell and a clonogenic thy-1 lo hematopoietic stem cell. Cell

44:653-662.
3. H. Kjetil et al. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. 1991. J. Immunol. 147: 22-28.
4. Brummer, Elmer et al. Immunological activation of polymorphonuclear neutrophils for fungal killing: Studies with murine cells and blastomyces dermatitidis in vitro. J. Leuko. Bio 36:505-520, 1984.
5. Jutila, M.A. et al. Ly-6C is a monocyte/macrophage and endothelial cell differentiation antigen regulated by interferon-gamma. J. Immunol. 18: 1819-1826, 1988.
6. Haestdal, K., F.W. Ruscetti, J.N. Ihle, S.E.W. Jacobsen, C.M. Dubois, W.C. Kopp, D.L. and J.R. Keller 1991. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cell. J.Immunol. 147:22
7. Fleming, T.J., M.L. Fleming, and T.R. Malek. 1993. Selective Expression of Ly-6G on myeloid lineage cells in bone marrow. RB6-85 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. J Immunol. 151:139

Protocols:

FLOW CYTOMETRY ANALYSIS:

Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population.
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 - 0.5 µg of antibody 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).

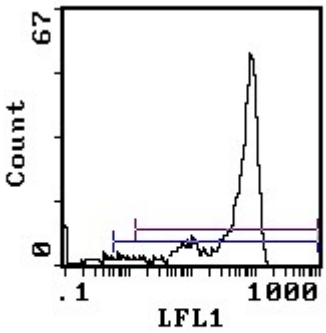
Results:
Tissue Distribution by Flow Cytometry Analysis:
Mouse Strain: CBA/J
Cell Concentration : 1x10e6 cells per test
Antibody Concentration Used: 0.1 µg/10e6 cells
Isotypic Control: FITC Rat IgG2b
Cell Source Percentage of cells stained above control:
Thymus 1.5%
Whole Blood Monocytes 87.2%
Bone Marrow Macrophages 90.0%

(see picture below)

Strain Distribution by Flow Cytometry Analysis:
Procedure: see above

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

Pictures:



Cell Source: Bone Marrow Macrophages
Percentage of cells stained above control: 90.0%