Anti-Gr-1 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
Anti-Gr-1-FITC	9 µg in 300 µL	130-102-837
Anti-Gr-1-FITC	30 µg in 1 mL	130-102-338
Anti-Gr-1-PE	9 µg in 300 µL	130-102-836
Anti-Gr-1-PE	30 µg in 1 mL	130-102-426
Anti-Gr-1-APC	9 µg in 300 µL	130-102-838
Anti-Gr-1-APC	30 µg in 1 mL	130-102-385
Anti-Gr-1-VioBlue	9 µg in 300 µL	130-102-830
Anti-Gr-1-VioBlue	30 µg in 1 mL	130-102-233
Anti-Gr-1-VioGreen	9 µg in 300 µL	130-102-835
Anti-Gr-1-VioGreen	30 µg in 1 mL	130-102-140
Anti-Gr-1-PerCP	9 µg in 300 µL	130-102-839
Anti-Gr-1-PerCP	30 µg in 1 mL	130-102-141
Anti-Gr-1-PerCP-Vio700	30 µg in 1 mL	130-102-171
Anti-Gr-1-Biotin	9 µg in 300 µL	130-101-997
Anti-Gr-1-Biotin	30 µg in 1 mL	130-101-894

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Technical data and background information

Antigen	Gr-1
Clone	RB6-8C5
Isotype	rat IgG2bκ
Isotype control	Rat IgG2b – isotype control antibodies
Alternative names of antigen	Ly-6G
Molecular mass of antigen [kDa]	10
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

The mouse Anti-Gr-1 antibody reacts with Ly-6G, a 21-25 kDa, GPI-anchored cell surface protein,

previously defined as the granulocyte-differentiation antigen-1 (Gr-1).[1] The antibody has been shown to cross-react weakly with Ly-6C-transfected EL-4J cells.[2] Cross-reactivity of Anti-Gr-1 with Ly-6C was not detected on hematopoietic cells that express Ly-6C and are negative for Ly-6G.[3]
Gr-1 is expressed on mature granulocytes in bone marrow and peripheral tissues. The Anti-Gr-1 antibody also stains monocytes transiently during their differentiation in bone marrow and at low levels plasmacytoid dendritic cells in lymphoid tissues.
When using formaldehyde-fixed cells that express Gr-1 at high levels, for example, granulocytes, the dilution is 1:50 for up to 10^7 cells/100 µL of buffer for labeling of cells and analyzing by flow cytometry.

Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C).
  Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for cell surface staining

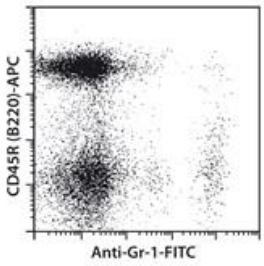
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

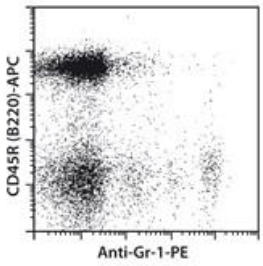
Examples of immunofluorescent staining

Mouse spleen cells were stained with Anti-Gr-1 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), PerCP-Vio700 (G) or Biotin (H) and CD45R (B220) and analyzed by flow cytometry. Cells stained with Anti-Gr-1-Biotin were stained with Anti-Biotin-APC in addition. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of Anti-Gr-1-Per-Vio700.

A:



B:



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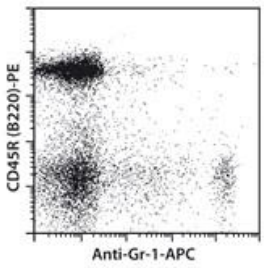

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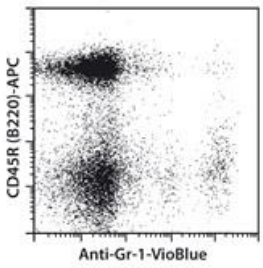

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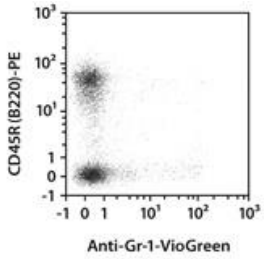

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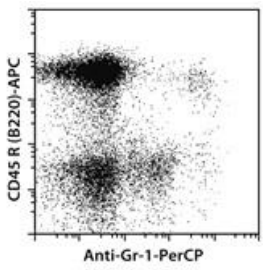

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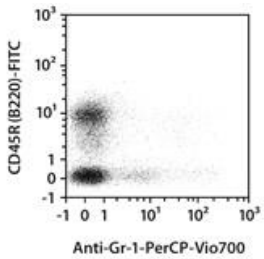

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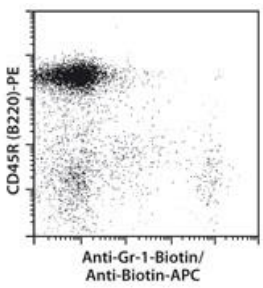

References

1. **Hestdal *et al.*** (1991) J. Immunol. 147: 22–28.
2. **Fleming *et al.*** (1993) J. Immunol. 151: 2399–2408.
3. **Nagendra *et al.*** (2004) Absence of cross-reactivity between murine Ly-6C and Ly-6G. Cytometry A 58: 195–200.

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