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1. Description

Components	1 mL monoclonal Anti-Gr-1 antibodies, mouse conjugated to various dyes.	
	FITC	130-091-934
	PE	130-091-932
	APC	130-091-931
	VioBlue®	130-094-362
	VioGreen™	130-097-292
	PerCP	130-094-958
	Biotin	130-091-930
Clone	RB6-8C5 (isotype: rat IgG2b).	
Capacity	100 tests or up to 10 ⁹ total cells.	
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.	
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.	

1.1 Background information

The mouse Anti-Gr-1 antibody reacts with Ly-6G, a 21–25 kDa GPI-anchored cell surface protein, previously defined as the myeloid differentiation antigen (Gr-1)¹. The antibody has also been shown to cross-react weakly with Ly-6C^{high} cells.² Gr-1 is expressed on mature granulocytes in bone marrow and peripheral tissues. It is also expressed transiently during monocyte differentiation in bone marrow and at low levels on plasmacytoid dendritic cells in lymphoid tissues.

1.2 Applications

- Identification and enumeration of Gr-1⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse plasmacytoid dendritic cells can be isolated by using, for example, the Plasmacytoid Dendritic Cell Isolation Kit II (# 130-092-786) or Anti-mPDCA-1 MicroBeads (# 130-091-965).

1.3 Recommended antibody dilution

The recommended antibody dilution for all Gr-1 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

When using formaldehyde-fixed cells that express Gr-1 at high levels, for example, granulocytes, the dilution is **1:50 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analyzing by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells.

1.4 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). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., APC (# 130-090-856) as secondary antibody reagent in combination with Anti-Gr-1-Biotin.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD45R (B220)-PE (# 130-091-828) or CD45R (B220)-APC (# 130-091-843). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

2. General protocol for immunofluorescent staining

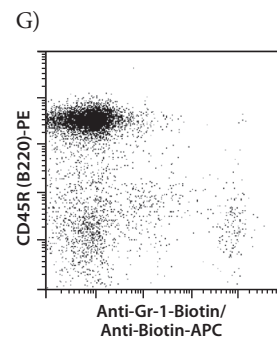
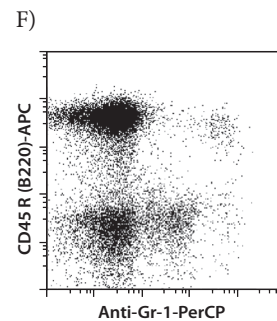
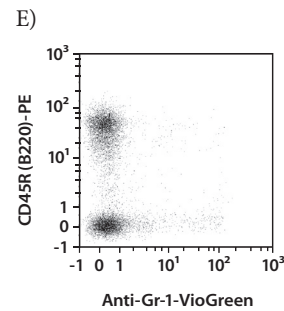
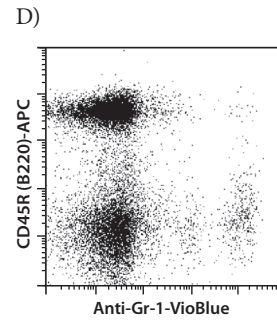
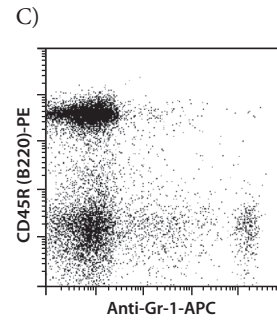
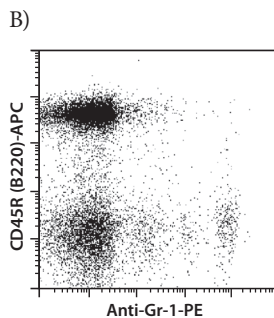
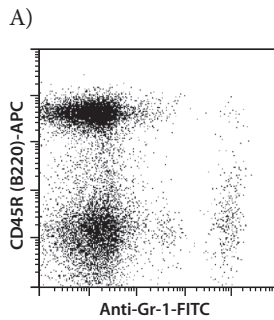
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 10⁷ nucleated cells per 100 μL of buffer.
4. Add 10 μL of the Anti-Gr-1 antibody.
 - ▲ **Note:** Refer to section 1.3 for exceptions.
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 Anti-Gr-1-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of 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.

3. Examples of immunofluorescent staining with Anti-Gr-1 antibodies

Mouse spleen cells were stained with Anti-Gr-1 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), or PerCP (F) as well as with CD45R (B220)-APC (# 130-091-843) or CD45R (B220)-PE (# 130-091-828) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-Gr-1-Biotin (G) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD45R (B220)-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Hestdal, K. *et al.* (1991) Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. *J. Immunol.* 147: 22–28.
2. Fleming, T. J. *et al.* (1993) Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. *J. Immunol.* 151: 2399–24087.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

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