



Anti-Ly-6G antibodies mouse

Anti-Ly-6G-FITC	130-093-138
Anti-Ly-6G-PE	130-093-139
Anti-Ly-6G-APC	130-093-140
Anti-Ly-6G-Biotin	130-093-141

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1. Description

Components	1 mL Anti-Ly-6G antibodies, mouse: monoclonal Anti-Ly-6G antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	1A8 (isotype: rat IgG2a).
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 Ly-6G antigen belongs to the Ly-6 family of murine surface glycoproteins.¹ Ly-6G is highly expressed on mature granulocytes. The expression greatly increases during maturation.^{2,3} To a lower extent, Ly-6G is also expressed during the development of monocytes.² In bone marrow, the Anti-Ly-6G antibody (clone 1A8) primarily detects granulocytes, whereas it does not recognize lymphocytes or erythrocytes³. Therefore, it represents a suitable tool for the analysis of granulocytes.

1.2 Applications

- Identification and enumeration of Ly-6G⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Ly-6G⁺ cells can be isolated by using the Anti-Ly-6G MicroBead Kit, mouse (# 130-092-332).

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-Ly-6G conjugate	FITC	PE	APC	Biotin
Flow cytometry^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10^7 cells/100 μ L of buffer.

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. 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-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-Ly-6G-Biotin.
- (Optional) CD11b-APC (# 130-091-241) or CD11b-PE (# 130-091-240).
- (Optional) Propidium iodide (PI) 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 10^7 nucleated cells. When working with fewer than 10^7 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 2×10^7 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 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the Anti-Ly-6G antibody.

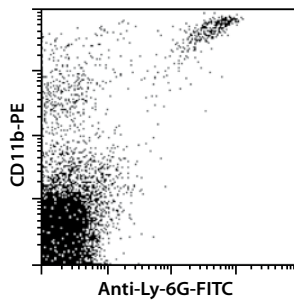


5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-Ly-6G-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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.

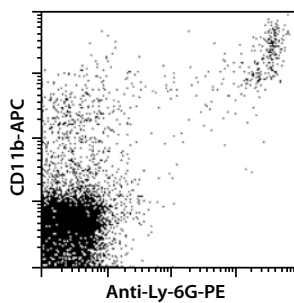
3. Examples of immunofluorescent staining with Anti-Ly-6G antibodies

Mouse spleen cells were stained with Anti-Ly-6G antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Cells labeled with Anti-Ly-6G-Biotin (d) were stained with Anti-Biotin-PE (# 130-090-756). In addition cells were counterstained with CD11b-APC or CD11b-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

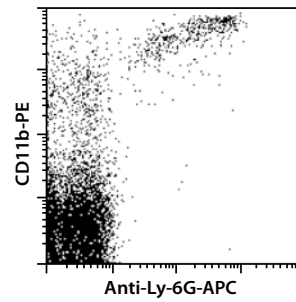
(a) Mouse spleen cells stained with Anti-Ly-6G-FITC and CD11b-PE.



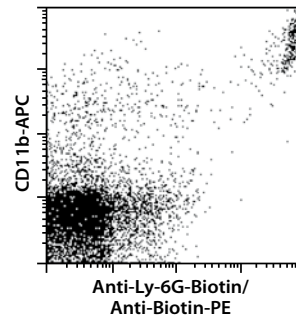
(b) Mouse spleen cells stained with Anti-Ly-6G-PE and CD11b-APC.



(c) Mouse spleen cells stained with Anti-Ly-6G-APC and CD11b-PE.



(d) Mouse spleen cells stained with Anti-Ly-6G-Biotin, Anti-Biotin-PE, and CD11b-APC.



4. References

1. Shevach, E. M. and Korty, P. E. (1989) Ly-6: a multigene family in search of a function. *Immunol. Today* 10: 195–220.
2. Hestdal, K. *et al.* (1991) Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. *J. Immunol.* 147: 22–28.
3. Fleming, T. J. *et al.* (1993) Selective expression of Ly-6G on myeloid lineage cells in bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J. Immunol.* 151: 2399–2408.

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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