

# Anti-Ly-6G antibodies, mouse

**For research use only**

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10<sup>6</sup> cells.

Product	Content	Order no.
Anti-Ly-6G-FITC	9 µg in 300 µL	130-102-934
Anti-Ly-6G-FITC	30 µg in 1 mL	130-102-296
Anti-Ly-6G-PE	9 µg in 300 µL	130-102-895
Anti-Ly-6G-PE	30 µg in 1 mL	130-102-392
Anti-Ly-6G-APC	9 µg in 300 µL	130-102-936
Anti-Ly-6G-APC	30 µg in 1 mL	130-102-342
Anti-Ly-6G-VioBlue	9 µg in 300 µL	130-103-136
Anti-Ly-6G-VioBlue	30 µg in 1 mL	130-102-227
Anti-Ly-6G-PerCP-Vio700	9 µg in 300 µL	130-103-861
Anti-Ly-6G-PerCP-Vio700	30 µg in 1 mL	130-103-791
Anti-Ly-6G-Biotin	30 µg in 1 mL	130-101-884

## 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

<b>Antigen</b>	Ly-6G
<b>Clone</b>	1A8
<b>Isotype</b>	rat IgG2ak
<b>Isotype control</b>	Rat IgG2a – isotype control antibodies
<b>Alternative names of antigen</b>	Gr-1
<b>Molecular mass of antigen [kDa]</b>	10
<b>Distribution of antigen</b>	neutrophils
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Fixation</b>	The antibody is suited for staining of formaldehyde-fixed cells.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze.

Ly-6G is highly expressed on neutrophilic granulocytes and expression greatly increases during granulocyte maturation.<sup>1</sup> To a lower extent, Ly-6G is also expressed during the development of monocytes. In mouse bone marrow samples, clone 1A8 primarily detects granulocytes, but not lymphocytes or erythrocytes.<sup>1</sup>

## Reagent requirements

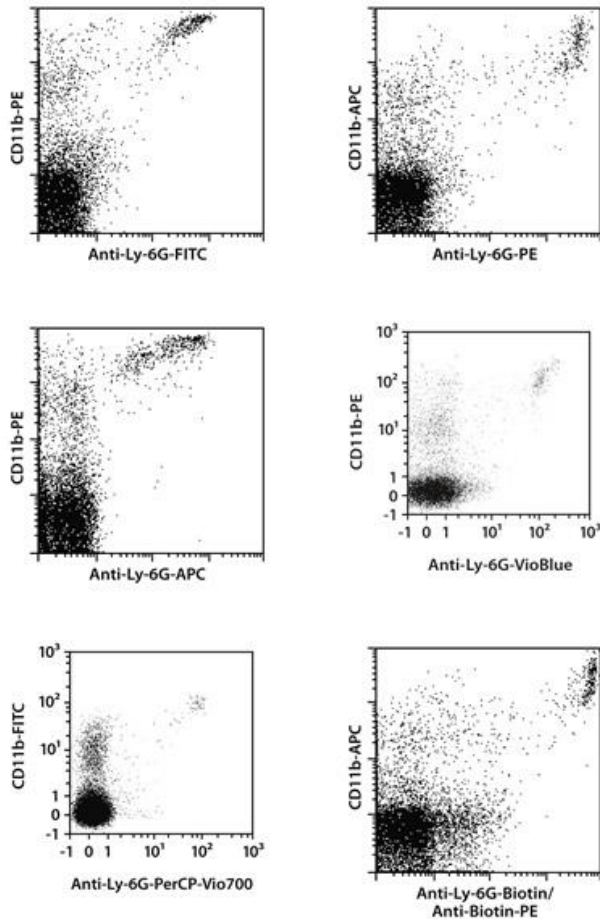
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> 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<sup>6</sup> cells/50 µL of buffer.
  - Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</sup> 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<sup>6</sup> 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<sup>6</sup> 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 splenocytes were stained with Anti-Ly-6G antibodies, as well as with CD11b antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## References

1. Fleming *et al.* (1993) J. Immunol. 151: 2399–2408.

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