



Anti-Ly-6C antibodies mouse

Anti-Ly-6C-FITC	130-093-134
Anti-Ly-6C-PE	130-093-135
Anti-Ly-6C-APC	130-093-136
Anti-Ly-6C-Biotin	130-093-137

Index

1. Description
 - 1.1 Background and product applications
 - 1.2 Recommended antibody dilution
 - 1.3 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with Anti-Ly-6C antibodies
4. References

1. Description

Clone	1G7.G10 (isotype: rat IgG2a).
Product format	1 mL Anti-Ly-6C antibodies, mouse: monoclonal Anti-Ly-6C antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests or up to 10 ⁹ total cells.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The Ly-6C antigen belongs to the Ly-6 family of murine surface glycoproteins¹. It is expressed on monocytes in the bone marrow and shortly after their migration into the circulation.² Ly-6C⁺ monocytes in the circulation have the capacity to migrate into sites of peripheral inflammation.² Crosslinking of Ly-6C is thought to mediate homing of Ly-6C⁺CD8⁺ T cells.³ Additionally, Ly-6C is expressed on memory T cells^{4,5} and on CD11^{low}CD45R(B220)⁺ mPDCA-1⁺ plasmacytoid DCs (PDCs) in different organs. Thus, in combination with Anti-mPDCA-1 antibodies, Anti-Ly-6C antibodies can be used for the identification of mouse plasmacytoid dendritic cells (PDCs).

Product applications

- Identification and enumeration of Ly-6C⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse PDCs can be isolated by using, for example, the Plasmacytoid Dendritic Cell Isolation Kit II (# 130-092-786), CD11c MicroBeads (# 130-052-001), Pan DC MicroBeads (# 130-092-465), or Anti-mPDCA-1 MicroBeads (# 130-091-965).

1.2 Recommended antibody dilution

For antibody labeling of mouse cells.

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

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1.3 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 (4–8 °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 calf 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-6C-Biotin.
- (Optional) Anti-mPDCA-1-FITC (# 130-091-961), Anti-mPDCA-1-PE (# 130-091-962), Anti-mPDCA-1-APC (# 130-091-963), or Anti-mPDCA-1-Biotin (# 130-091-964).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling 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. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
2. Add 10 µL of the Anti-Ly-6C antibody.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).
▲ Note: Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

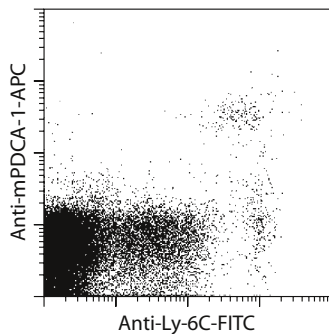


- (Optional) If Anti-Ly-6C-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in steps 3 and 4.
- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

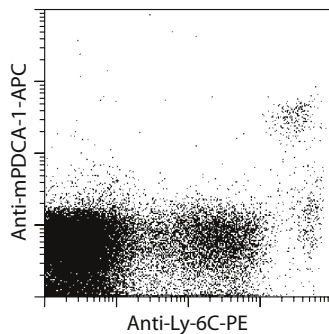
3. Examples of immunofluorescent staining with Anti-Ly-6C antibodies

Mouse splenocytes were stained with Anti-Ly-6C antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Cells stained with Anti-Ly-6C-Biotin (d) were stained with Anti-Biotin-FITC (# 130-090-857). Additionally, cells were stained with Anti-mPDCA-1-APC or Anti-mPDCA-1-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

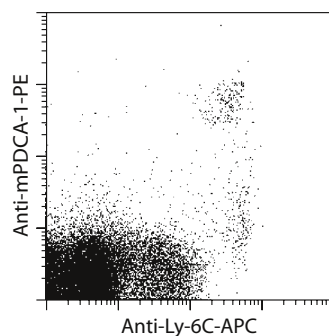
(a) Mouse splenocytes stained with Anti-Ly-6C-FITC and Anti-mPDCA-1-APC.



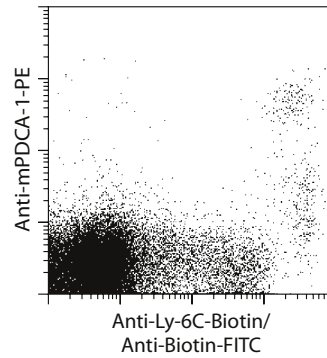
(b) Mouse splenocytes stained with Anti-Ly-6C-PE and Anti-mPDCA-1-APC.



(c) Mouse splenocytes stained with Anti-Ly-6C-APC and Anti-mPDCA-1-PE.



(d) Mouse splenocytes stained with Anti-Ly-6C-Biotin, Anti-Biotin-FITC, and Anti-mPDCA-1-PE.



4. References

- Shevach, E. M. and Korty, P. E. (1989) Ly-6: a multigene family in search of a function. *Immunol. Today* 10: 195–220.
- Sunderkötter, C. *et al.* (2004) Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J. Immunol.* 172: 4410–4417.
- Hänninen, A. *et al.* (1997) Ly-6C regulates endothelial adhesion and homing of CD8⁺ T cells by activating integrin-dependent adhesion pathways. *Proc. Natl. Acad. Sci. USA* 94: 6898–6903.
- Curtsinger, J. M. *et al.* (1998) CD8⁺ memory T cells (CD44^{high}, Ly-6C⁺) are more sensitive than naive cells to (CD44^{low}, Ly-6C⁻) to TCR/CD8 signaling in response to antigen. *J. Immunol.* 160: 3236–3243.
- Schlueter, A. J. *et al.* (1997) Distribution of Ly-6C on lymphocyte subsets: I. Influence of allotype on T lymphocyte expression. *J. Immunol.* 158: 4211–4222.

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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140602-07401

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