

# CD127 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.
CD127-VioBright FITC	9 μg in 300 μL	130-105-168
CD127-VioBright FITC	30 μg in 1 mL	130-105-107
CD127-PE	9 μg in 300 μL	130-102-984
CD127-PE	30 μg in 1 mL	130-102-551
CD127-APC	9 μg in 300 μL	130-102-847
CD127-APC	30 μg in 1 mL	130-102-529
CD127-PE-Vio770	9 μg in 300 μL	130-106-150
CD127-PE-Vio770	30 μg in 1 mL	130-106-099
CD127-Biotin	9 μg in 300 μL	130-102-039
CD127-Biotin	30 μg in 1 mL	130-101-910

# 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
 CD127

 Clone
 A7R 34

 Isotype
 rat IgG2ak

**Isotype control** Rat IgG2a – isotype control antibodies

Alternative names of antigen IL-7R, IL-7Ra

Molecular mass of antigen [kDa] 50

**Distribution of antigen**B cells, bone marrow, liver, lymphocytes, monocytes, T cells,

thymocytes

Product format Antibodies are supplied in buffer containing stabilizer and 0.05%

sodium azide.

**Fixation** Cells should be stained prior to fixation, if formaldehyde is used

as a fixative.

**Storage** Store protected from light at 2–8 °C. Do not freeze.

The antibody clone A7R34 reacts with CD127, also known as IL-7 receptor  $\alpha$  chain (IL-7R $\alpha$ ). Mouse CD127 is a 60-90 kDa type-I transmembrane glycoprotein. It is a component of the high affinity IL-7 receptor (IL-7R) and the receptor for thymic stromal lymphopoietin (TSLP)<sup>1</sup>. CD127 is expressed on

immature B cells, CD4<sup>-</sup>CD8<sup>-</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> thymocytes, naive and memory T cells, thymic NK cells, and bone marrow stromal cells.<sup>2,3</sup> Upon activation of naive T cells, CD127 expression is downregulated. Re-expression of CD127 identifies the effector cells that will differentiate into memory T cells.<sup>4</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

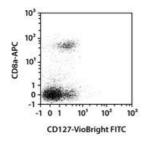
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to  $10^6$  cells/50 µL of buffer.
- Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</sup> cells, use the same volumes as indicated. 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  $10^6$  nucleated cells per 45  $\mu$ L 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).

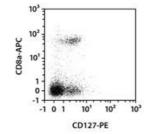
  Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

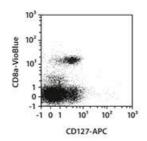
  Working on ice requires increased incubation times.
- 6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
- 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of fluorochrome-conjugated 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.

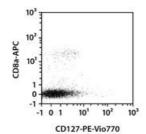
### **Examples of immunofluorescent staining**

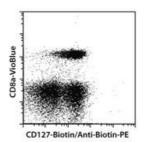
Mouse splenocytes were stained with CD127 antibodies as well as with CD8a antibodies. Cells stained with CD127-Biotin were also stained with Anti-Biotin-PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.











#### References

- 1. **Goodwin, R. G. et al.** (1990) Cloning of the human and murine interleukin-7 receptors: demonstration of a soluble form and homology to a new receptor superfamily. Cell 60: 941–951.
- Sudo, T. et al. (1993) Expression and function of the interleukin 7 receptor in murine lymphocytes. Proc. Natl. Acad. Sci. U.S.A. 90: 9125–9129.
- 3. Vosshenrich, C. A. et al. (2006) A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127. Nat. Immunol. 7: 1217–1224.
- 4. **Kaech, S. M.** et al. (2003) Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat. Immunol. 4: 1191–1198.
- Colpitts, S. L. et al. (2009) IL-7 receptor expression provides the potential for long-term survival of both CD62L<sup>high</sup> central memory T cells and TH1 effector cells during *Leishmania major* infection. J. Immunol. 182: 5702–5711.

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Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com | Miltenyi Biotec provides products and services worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec

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