

CD127-PE	130-094-577
CD127-Biotin	130-094-583
CD127 pure – functional grade	130-094-828

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## 1. Description

<b>Components</b>	1 mL CD127 antibodies, mouse: monoclonal CD127 antibodies conjugated to R-phycoerythrin (PE) or biotin. The unconjugated (pure) antibody is supplied at a concentration of 1 mg in 0.5 mL.
<b>Clone</b>	A7R34 (isotype: rat IgG2a).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.  The functional grade antibody is supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/μg of protein.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

*The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.*

### 1.1 Background information

Mouse CD127, also known as IL-7 receptor  $\alpha$  chain (IL-7R $\alpha$ ), 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>1,2</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>3,4</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>5</sup>. The antibody clone A7R34 has been used *in vitro* and *in vivo* for blocking of IL-7 binding to its receptor<sup>3,6</sup>.

## 1.2 Applications

- Identification and enumeration of CD127<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- The CD127 pure – functional grade antibody is suited for functional assays, for example, blocking of receptor-ligand binding<sup>3,6</sup>.

## 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

CD127 conjugate	PE	Biotin
<b>Flow cytometry<sup>a</sup></b>		
- In general	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11

a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100  $\mu$ L of buffer.  
b) For optimal results, cells must be stained prior to fixation.

## 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<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). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum. 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) Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD127-Biotin.
- (Optional) Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD4-FITC (# 130-091-608), CD4-PE (# 130-091-607), CD4-APC (# 130-091-611), CD8a-FITC (# 130-091-605), CD8a-PE (# 130-091-603), CD8a-APC (# 130-094-606), or CD8a-VioBlue™ (# 130-094-360). For more information about fluorochrome-conjugated antibodies see [www.miltenyibiotec.com](http://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  $10^7$  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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

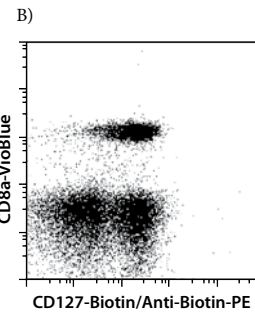
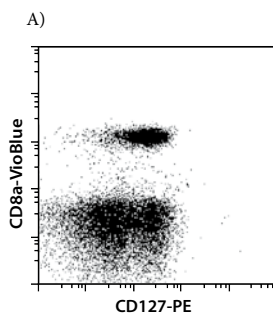
1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the CD127 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).

▲ **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 and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD127-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of anti-biotin antibody (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 CD127 antibodies

Mouse splenocytes were stained with CD127 antibodies conjugated to PE (A) as well as with CD8a-VioBlue (# 130-094-360) and analyzed using the MACSQuant® Analyzer. Cells labeled with CD127-Biotin (B) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD8a-VioBlue. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. 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.
2. Park, L. S. *et al.* (2000) Cloning of the Murine Thymic Stromal Lymphopoietin (TSLP) Receptor: Formation of a Functional Heteromeric Complex Requires Interleukin 7 Receptor. *J. Exp. Med.* 192: 659–669.
3. Sudo, T. *et al.* (1993) Expression and function of the interleukin 7 receptor in murine lymphocytes. *Proc. Natl. Acad. Sci. USA* 90: 9125–9129.
4. 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.
5. 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.
6. 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.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

### Warranty

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