

CD127 antibodies

human

| | |
|--------------|-------------|
| CD127-FITC | 130-094-888 |
| CD127-PE | 130-094-889 |
| CD127-APC | 130-094-890 |
| CD127-Biotin | 130-094-891 |
| CD127 pure | 130-094-942 |

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1. Description

| | |
|-----------------------|--|
| Components | 1 mL CD127 antibodies, human: monoclonal CD127 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. |
| Clone | MB15-18C9 (isotype: mouse 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

CD127, the α -chain of the IL-7 receptor, is a type I membrane glycoprotein. Signaling of IL-7 through the IL-7R requires both IL-7R α and the common cytokine gamma chain (γ c).¹ CD127 can be identified on immature B cells through the early pre-B stage, on thymocytes, and on most mature T cells with transient down-regulation upon activation.^{2,3} On regulatory T cells CD127 is absent⁴ and its expression is inversely correlated with FoxP3 expression and suppressive function.^{5,6} CD127 is also used by thymic stromal-derived lymphopoietin (TSLP) as part of a complex.¹

1.2 Applications

- Analysis of human regulatory T cells by using, for example, CD4-FITC (# 130-092-358), CD25-PE (# 130-091-024), and CD127-APC monoclonal antibodies
- Identification and enumeration of CD127⁺ cells by flow cytometry or fluorescence microscopy.
- Identification of memory CD8 T cell precursors within an effector T cell population.

- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human regulatory T cells can be isolated, for example, by using the CD4⁺CD25⁺CD127^{dim/-} Regulatory T Cell Isolation Kit, human (# 130-094-775).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

| CD127 conjugate | FITC | PE | APC | Biotin |
|---|------|------|------|--------|
| Flow cytometry^a | | | | |
| - In general | 1:11 | 1:11 | 1:11 | 1:11 |
| - Formaldehyde-fixed cells ^b | 1:11 | 1:11 | 1:11 | 1:11 |
| - CD127 MicroBead-labeled cells | 1:11 | 1:11 | 1:11 | 1:11 |

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

- **Cross-reactivity:** The CD127 antibody is tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

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 human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- **FcR Blocking Reagent, human** (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) **Anti-Biotin-FITC** (# 130-090-857), **Anti-Biotin-PE** (# 130-090-756), **Anti-Biotin-APC** (# 130-090-856), or **Anti-Biotin-VioBlue** (# 130-094-669) as secondary antibody reagent in combination with CD127-Biotin.
- (Optional) **CD4-PE, human** (# 130-091-231) or **CD4-APC, human** (# 130-091-232). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) **Mouse IgG2a-FITC** (# 130-091-837), **Mouse IgG2a-PE** (# 130-091-835), or **Mouse IgG2a-APC** (# 130-091-836) for isotype control.
- (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.

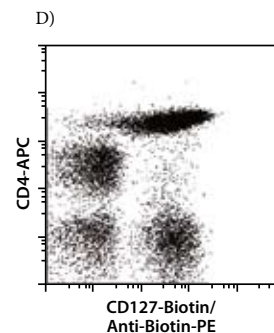
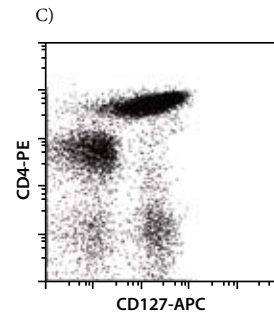
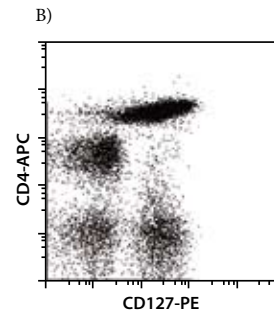
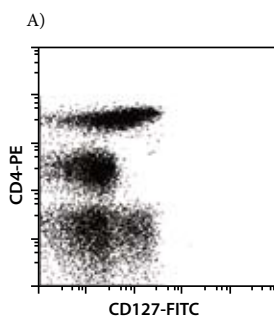
2. General protocol for immunofluorescent staining

▲ 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 2×10^7 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 90 μL of buffer.
4. Add 10 μL of FcR Blocking Reagent.
5. Mix well and incubate for 10 minutes 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. Add 10 μL of the CD127 antibody.
7. 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.
8. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
9. (Optional) If Anti-CD127-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 7 and 8.
10. 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

Human peripheral blood mononuclear cells (PBMCs) were stained with CD127 antibodies conjugated to FITC (A), PE (B), or APC (C), as well as with CD4-PE (# 130-091-231) or CD4-APC (# 130-091-232) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD127-Biotin (D) were stained with Anti-Biotin-PE (# 130-090-756) and CD4-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Fry, T.J. and Mackall, C.L. (2002) Interleukin-7: from bench to clinic. *Blood* 99: 3892–3904.
2. Sudo, T. *et al.* (1993) Expression and function of the interleukin 7 receptor in murine lymphocytes. *J. Immunol.* 90: 9125–9129.
3. Armitage, R.J. *et al.* (1991) Expression of receptors for interleukin 4 and interleukin 7 on human T cells. *Adv. Exp. Med. Biol.* 292: 121–130.
4. Cupedo, T. *et al.* (2005) Development and activation of regulatory T cells in the human fetus. *Eur. J. Immunol.* 35: 383–390.
5. Seddiki, N. *et al.* (2006) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J. Exp. Med.* 203: 1693–1700.
6. Liu, W. *et al.* (2006) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4^+ T reg cells. *J. Exp. Med.* 203: 1701–1711.

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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