CD127 antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD127-VioBright FITC	for 30 tests	130-109-518
CD127-VioBright FITC	for 100 tests	130-109-439
CD127-PE	for 30 tests	130-109-514
CD127-PE	for 100 tests	130-109-435
CD127-APC	for 30 tests	130-109-515
CD127-APC	for 100 tests	130-109-436
CD127-PE-Vio770	for 30 tests	130-109-516
CD127-PE-Vio770	for 100 tests	130-109-437
CD127-APC-Vio770	for 30 tests	130-109-517
CD127-APC-Vio770	for 100 tests	130-109-438

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	REA614
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	IL-7R, IL-7Rα, IL-17Ralpha, CDW127, ILRA
Molecular mass of antigen [kDa]	50
Distribution of antigen	T cells
Product format	Reagents 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.

Clone REA614 recognizes the CD127 antigen, which is the α-chain of the interleukin 7 (IL-7) receptor, a type I membrane glycoprotein. Signaling of IL-7 through the IL-7R requires both IL-7Rα and the common cytokine gamma chain (γc). 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. On regulatory T cells CD127 is absent and its expression is inversely correlated with FoxP3 expression

and suppressive function. CD127 is also used by thymic stromal derived lymphopoietin (TSLP) as part of a complex.
Additional information: Clone REA614 displays negligible binding to Fc receptors.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (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.

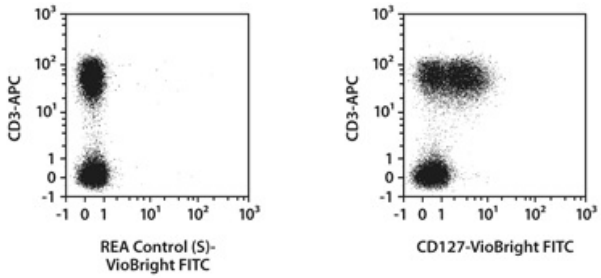
Protocol for cell surface staining

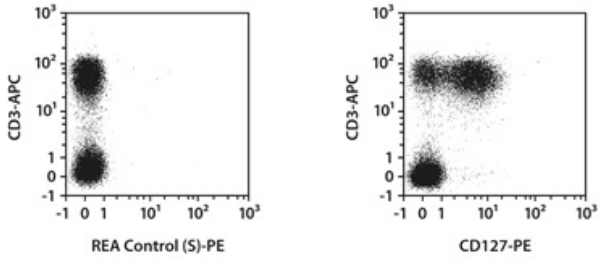
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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).

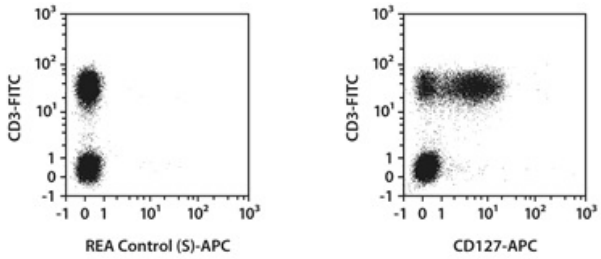
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

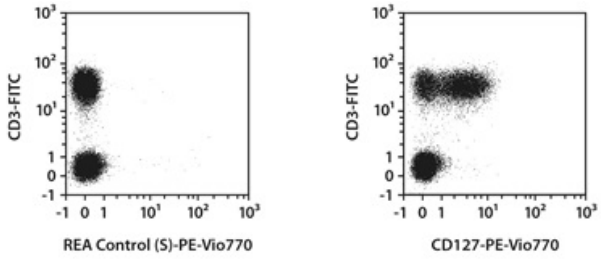
Examples of immunofluorescent staining

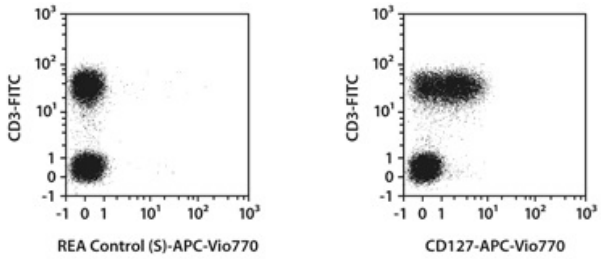
Human peripheral blood mononuclear cells (PBMCs) were stained with CD127 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.











References

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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.

Warranty

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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**
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