

# CD123 antibodies, human

For research use only

One test corresponds to labeling of up to  $10^7$  cells in a total volume of 100  $\mu$ L.

Product	Content	Order no.
CD123-FITC	for 30 tests	130-098-886
CD123-FITC	for 100 tests	130-090-897
CD123-PE	for 30 tests	130-098-894
CD123-PE	for 100 tests	130-090-899
CD123-APC	for 30 tests	130-098-873
CD123-APC	for 100 tests	130-090-901
CD123-VioBlue	for 30 tests	130-099-718
CD123-VioBlue	for 100 tests	130-097-330
CD123-VioGreen	for 30 tests	130-106-711
CD123-VioGreen	for 100 tests	130-106-654
CD123-PE-Vio615	for 30 tests	130-108-385
CD123-PE-Vio615	for 100 tests	130-108-356
CD123-PE-Vio770	for 30 tests	130-104-224
CD123-PE-Vio770	for 100 tests	130-104-191
CD123-APC-Vio770	for 30 tests	130-104-229
CD123-APC-Vio770	for 100 tests	130-104-196
CD123-PerCP-Vio700	for 30 tests	130-103-872
CD123-PerCP-Vio700	for 100 tests	130-103-802
CD123-Biotin	for 30 tests	130-098-566
CD123-Biotin	for 100 tests	130-098-565
CD123 pure	50 $\mu$ g in 1 mL	130-090-940

## 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

<b>Antigen</b>	CD123
<b>Clone</b>	AC145
<b>Isotype</b>	mouse IgG2ak
<b>Isotype control</b>	Mouse IgG2a – isotype control antibodies
<b>Alternative names of antigen</b>	IL3RA, IL3R, IL3RAY, IL3RX, IL3RY, hIL-3Ra, IL-3R $\alpha$
<b>Molecular mass of antigen [kDa]</b>	41

<b>Distribution of antigen</b>	B cells, basophils, bone marrow, brain, endothelial cells, eosinophils, granulocytes, leukemia cells, macrophages, mast cells, megakaryocytes, monocytes, myeloid cells, placenta, red blood cells
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Fixation</b>	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze.

CD123 is also known as IL-3 receptor  $\alpha$ -chain and is the primary low-affinity subunit of the IL-3 receptor. CD123 associates with CD131, the common  $\beta$ -chain of the IL-3, IL-5, and GM-CSF receptor, to form the high-affinity IL-3 receptor. The IL-3 receptor is involved in cell signaling for cell growth and differentiation. In peripheral blood, the CD123 antigen is expressed at high levels only on plasmacytoid dendritic cells and basophilic granulocytes but at low levels also on monocytes, eosinophilic granulocytes, myeloid dendritic cells, and subsets of hematopoietic progenitor cells.

## Reagent requirements

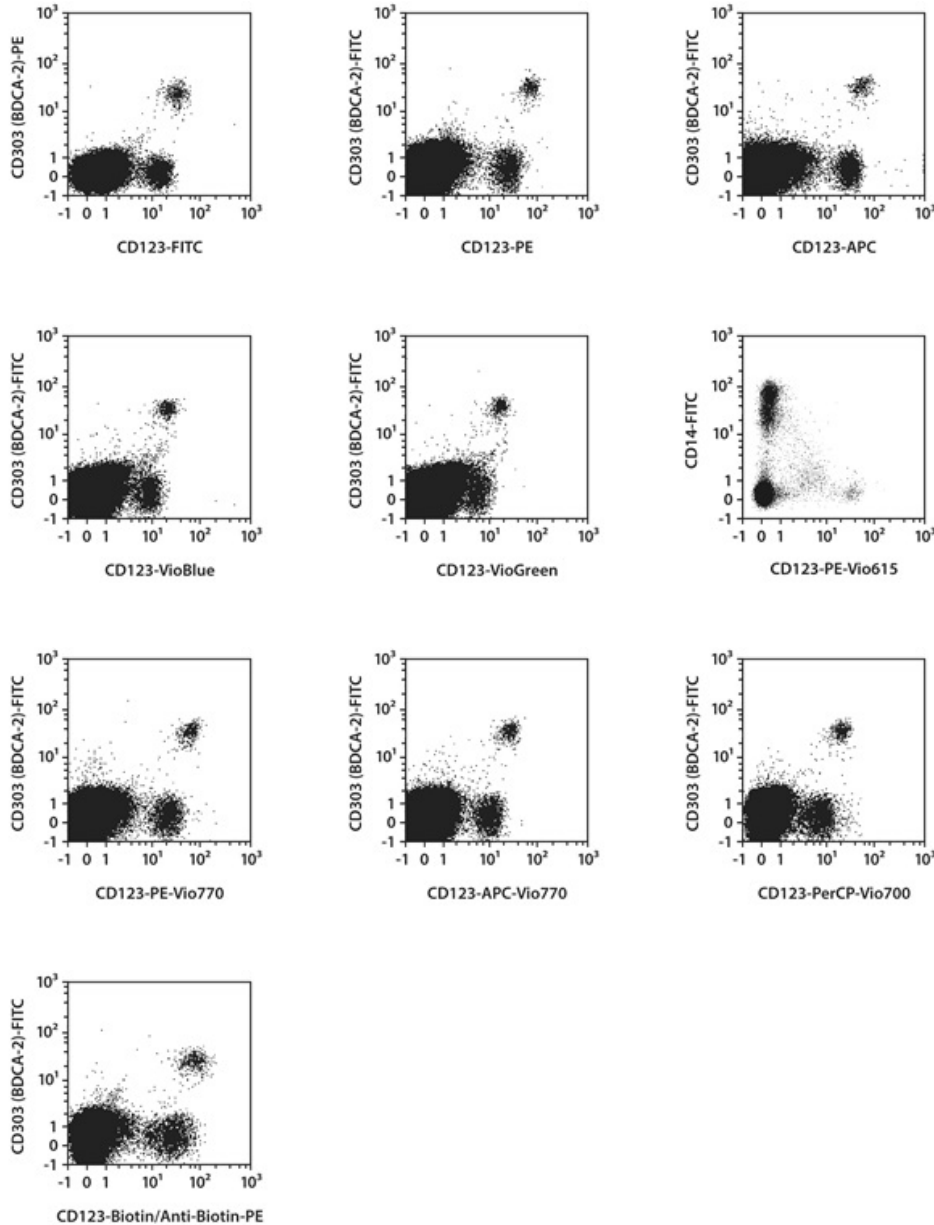
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can 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, human (# 130-059-901) 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 1:11 for up to 10<sup>7</sup> cells/100  $\mu$ L of buffer.
  - Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</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 $\times$ 10<sup>7</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 $\times$ g for 10 minutes. Aspirate supernatant completely.
  3. Resuspend up to 10<sup>7</sup> nucleated cells per 100  $\mu$ L of buffer.
  4. Add 10  $\mu$ L 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 $\times$ 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

Human peripheral blood mononuclear cells (PBMCs) were stained with CD123 antibodies as well as with CD303 (BDCA-2) antibodies and analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye-conjugated antibodies. For all other conjugates the FcR Blocking Reagent has been used to avoid Fc receptor-mediated antibody labeling. 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

1. **Choi, K.-D. et al.** (2011) Hematopoietic differentiation and production of mature myeloid cells from human pluripotent stem cells. *Nat. Protoc.* 6(3): 296–313.
2. **van Dongen, J. J. M. et al.** (2012) EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia* 26(9): 1908–1975.

## Warranty

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