CD123 antibodies, human

For research use only

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

Product	Content	Order no.
CD123-VioBlue	for 30 tests	130-115-363
CD123-FITC	for 30 tests	130-115-356
CD123-FITC	for 100 tests	130-115-263
CD123-PE	for 30 tests	130-115-357
CD123-PE	for 100 tests	130-115-264
CD123-APC	for 30 tests	130-115-358
CD123-APC	for 100 tests	130-115-265
CD123-VioBlue	for 100 tests	130-115-270
CD123-VioGreen	for 30 tests	130-115-364
CD123-VioGreen	for 100 tests	130-115-271
CD123-PE-Vio615	for 30 tests	130-115-365
CD123-PE-Vio615	for 100 tests	130-115-272
CD123-PE-Vio770	for 30 tests	130-115-359
CD123-PE-Vio770	for 100 tests	130-115-266
CD123-APC-Vio770	for 30 tests	130-115-360
CD123-APC-Vio770	for 100 tests	130-115-267
CD123-PerCP-Vio700	for 30 tests	130-115-361
CD123-PerCP-Vio700	for 100 tests	130-115-268
CD123-Biotin	for 30 tests	130-115-355
CD123-Biotin	for 100 tests	130-115-262
CD123-VioBright 667	for 30 tests	130-118-374
CD123-VioBright 667	for 100 tests	130-118-220

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 CD123

Clone REA918

Isotype recombinant human IgG1

Isotype control	REA Control (S) antibodies
Alternative names of antigen	IL3RA, IL3R, IL3RAY, IL3RX, IL3RY, hIL-3Ra, IL-3Rα
Entrez Gene ID	3563
Molecular mass of antigen [kDa]	41
Cross-reactivity	rhesus monkey (*Macaca mulatta*)
Distribution of antigen	dendritic cells, granulocytes, mast cells, megakaryocytes, monocytes, basophils, eosinophils, hematopoietic stem cells, myeloid cells, plasma 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 REA918 recognizes the human CD123 antigen, a type I transmembrane glycoprotein, which is also known as interleukin 3 (IL-3) receptor α-chain and is the primary low-affinity subunit of the IL-3 receptor. CD123 associates with CD131, the common β-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. Additional information: Clone REA918 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.

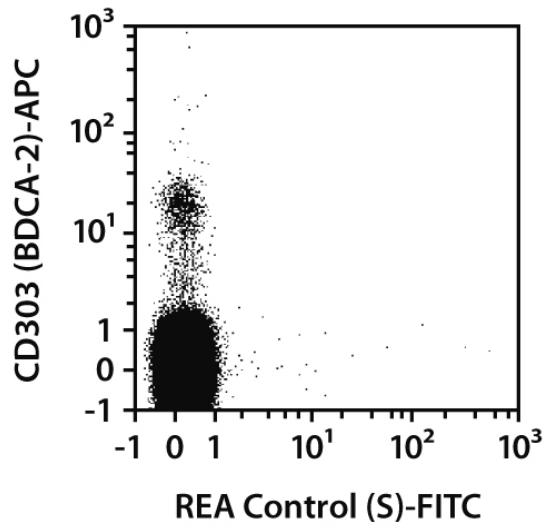
Protocol for cell surface staining

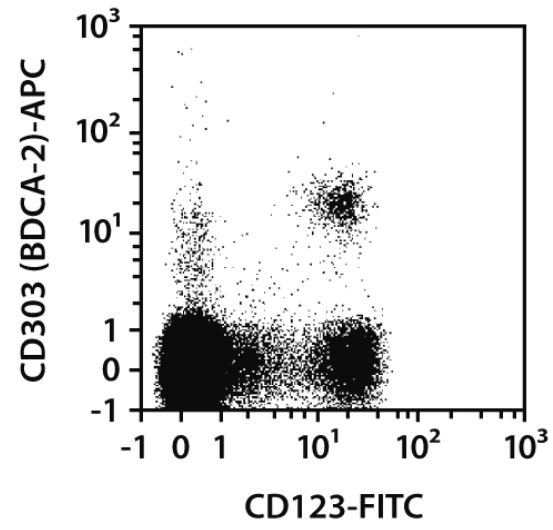
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

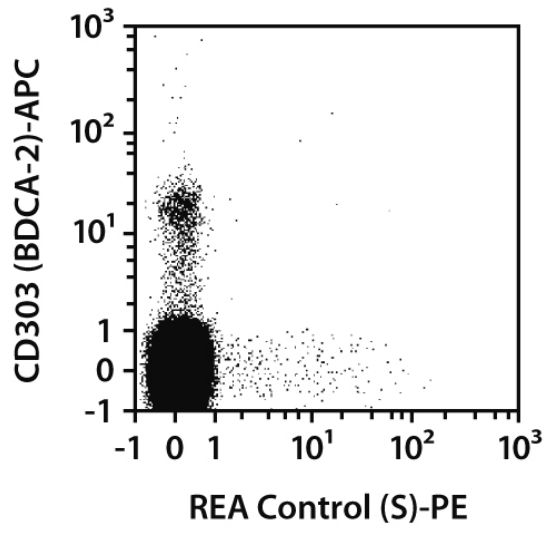
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

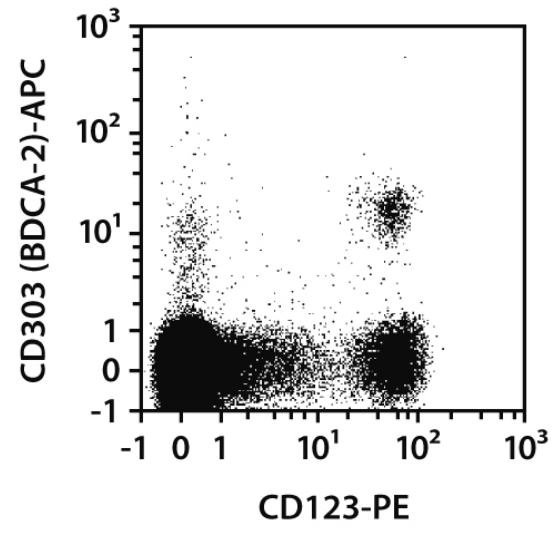
Examples of immunofluorescent staining

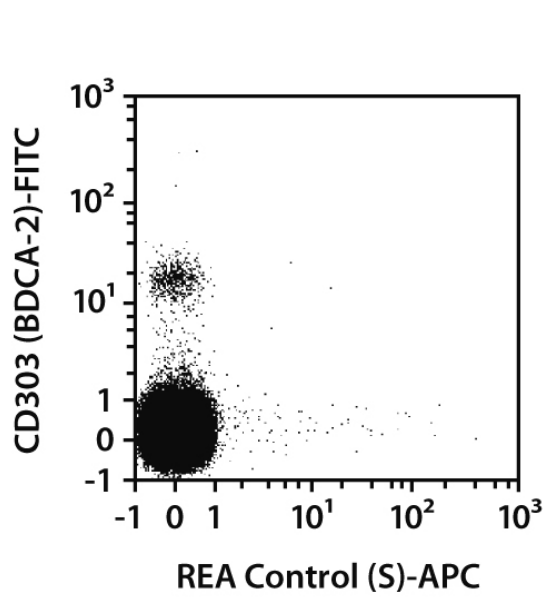
Human peripheral blood mononuclear cells (PBMCs) were stained with CD123 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD303 (BDACA-2) antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. The Tandem Signal Enhancer has been used to increase 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.

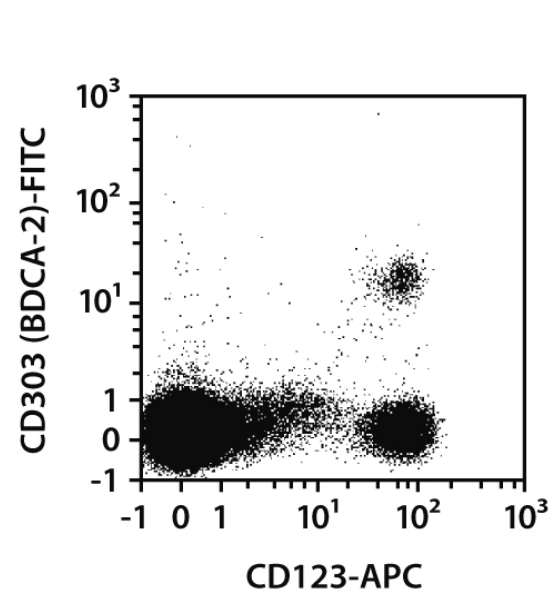


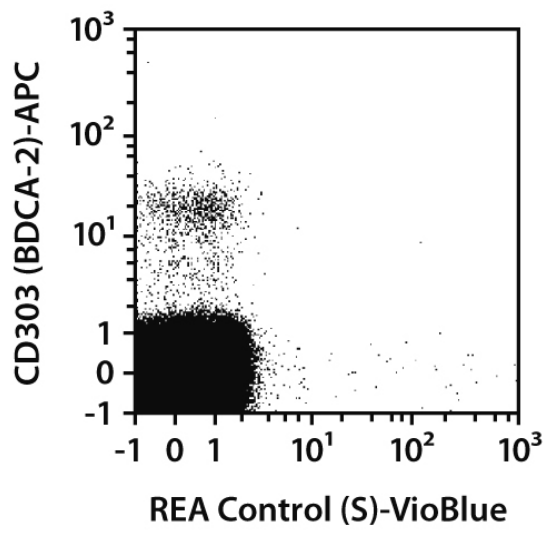


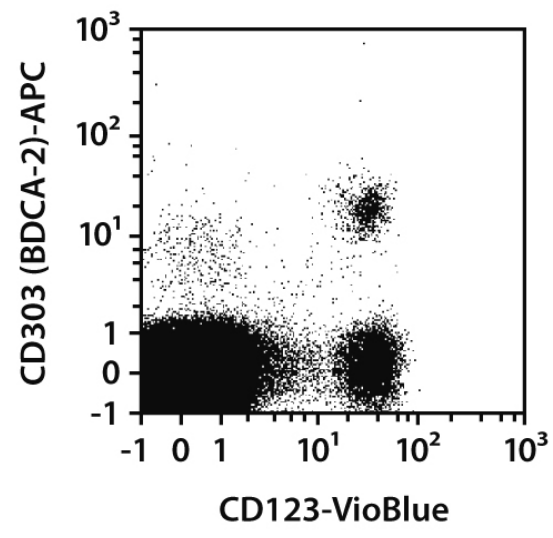


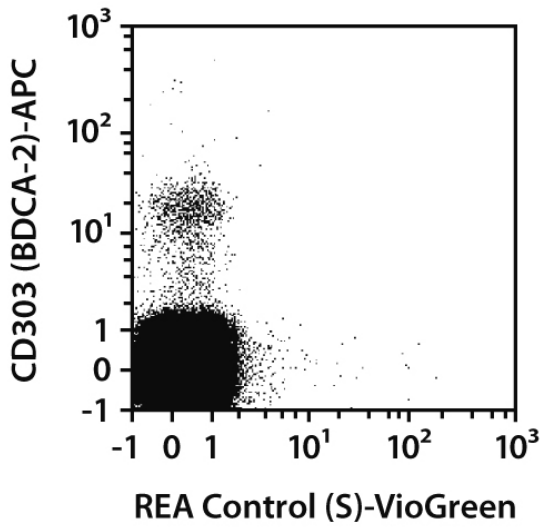


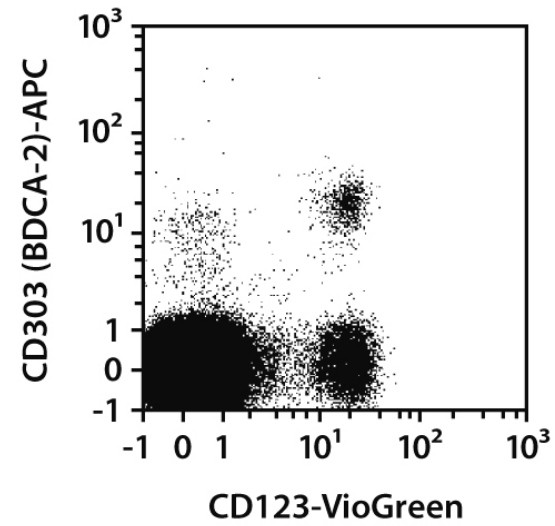









CD303 (BDCA-2)-APC
REA Control (S)-VioGreen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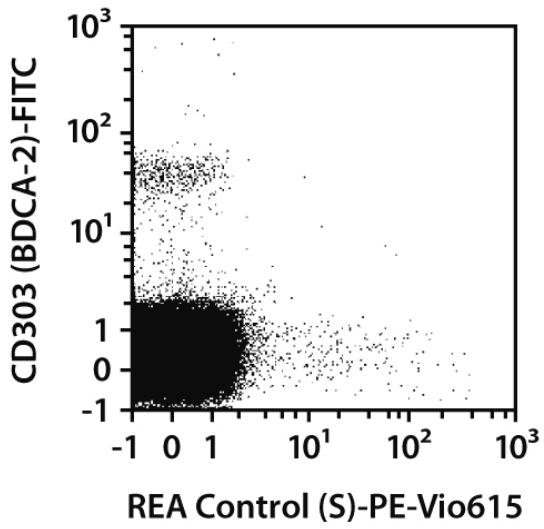
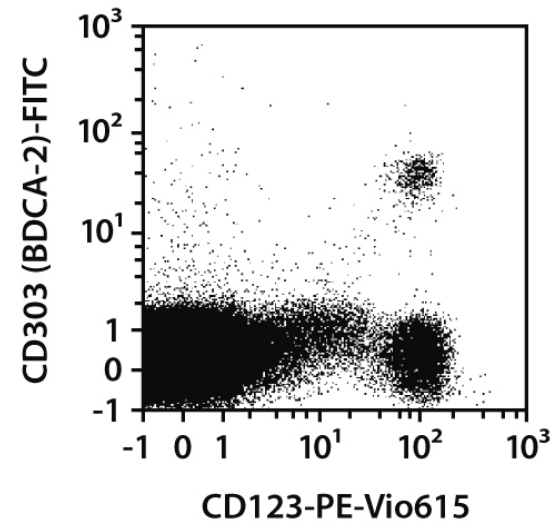
CD123-VioGreen


CD303 (BDCA-2)-FITC
REA Control (S)-PE-Vio615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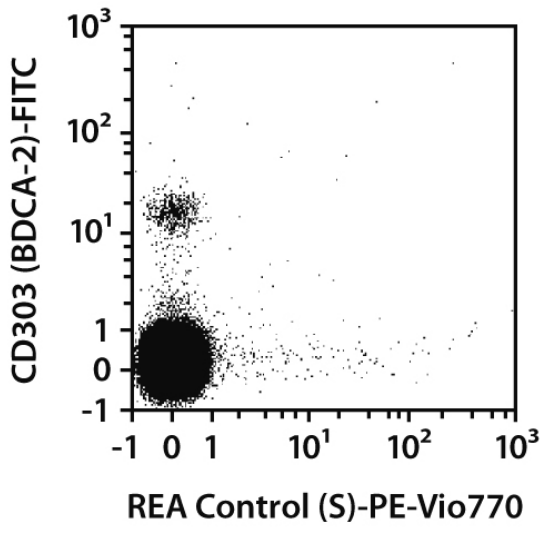
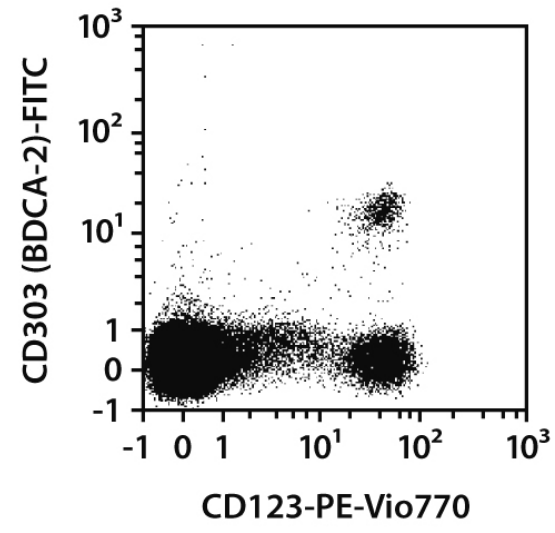
CD123-PE-Vio615


CD303 (BDCA-2)-FITC
REA Control (S)-PE-Vio770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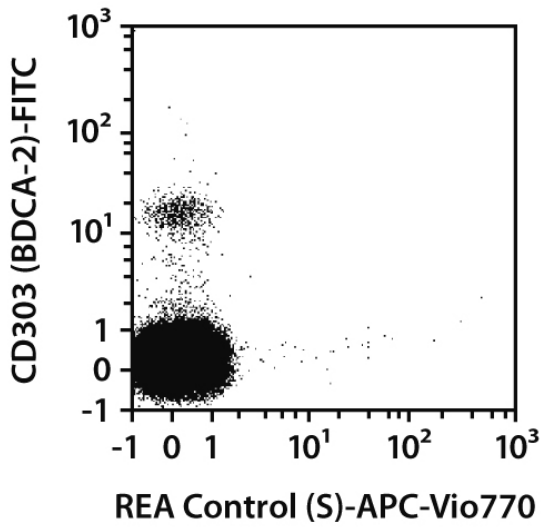
CD123-PE-Vio770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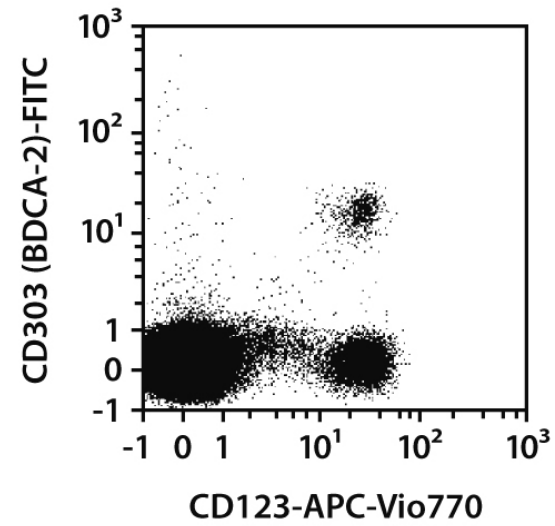
REA Control (S)-APC-Vio770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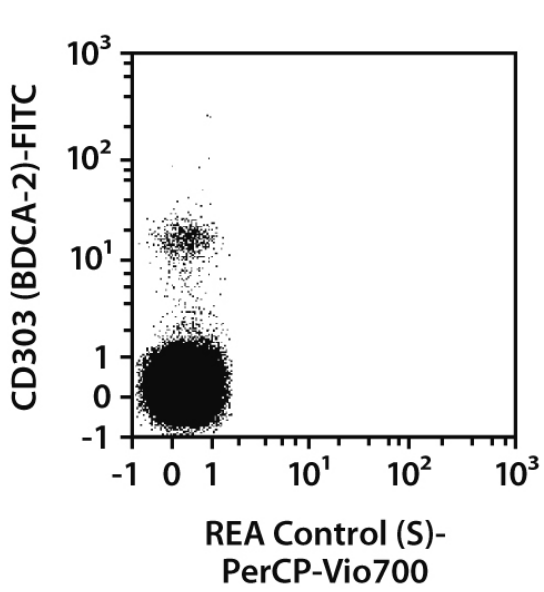
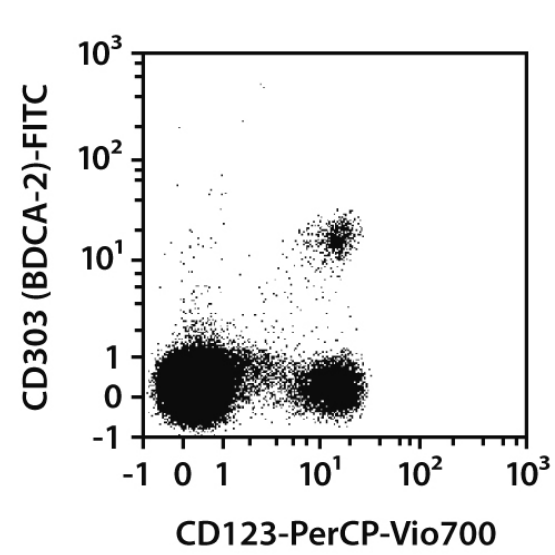
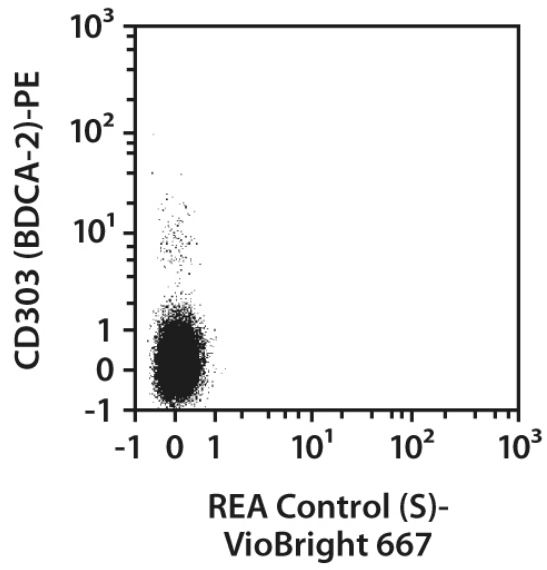
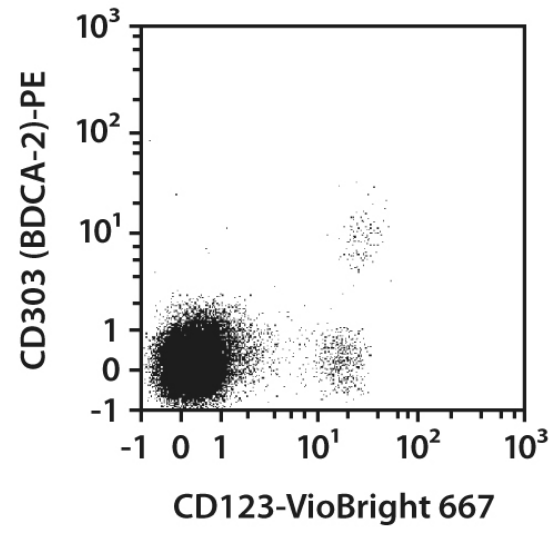
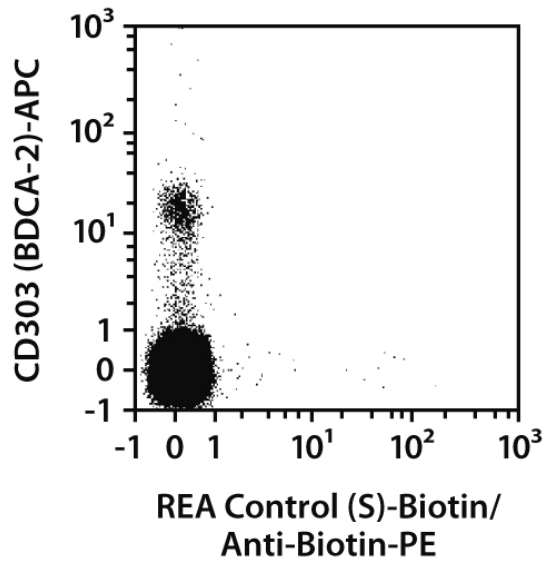
CD123-APC-Vio770

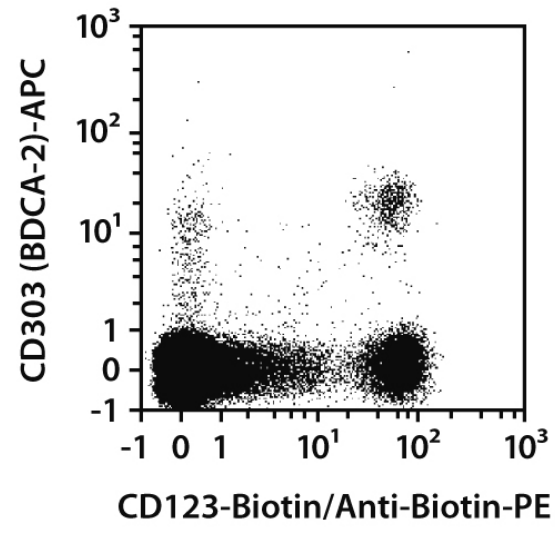

CD303 (BDCA-2)-FITC
REA Control (S)-
PerCP-Vio700


CD303 (BDCA-2)-FITC
CD123-PerCP-Vio700









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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 contact.