

CD141 (BDCA-3) antibodies, human

For research use only

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

Product	Content	Order no.
CD141 (BDCA-3)-VioBlue	for 30 tests	130-111-045
CD141 (BDCA-3)-FITC	for 30 tests	130-110-358
CD141 (BDCA-3)-FITC	for 100 tests	130-110-257
CD141 (BDCA-3)-PE	for 30 tests	130-110-359
CD141 (BDCA-3)-PE	for 100 tests	130-110-258
CD141 (BDCA-3)-APC	for 30 tests	130-110-360
CD141 (BDCA-3)-APC	for 100 tests	130-110-259
CD141 (BDCA-3)-VioBlue	for 100 tests	130-110-928
CD141 (BDCA-3)-PE-Vio770	for 30 tests	130-110-361
CD141 (BDCA-3)-PE-Vio770	for 100 tests	130-110-260
CD141 (BDCA-3)-APC-Vio770	for 30 tests	130-110-362
CD141 (BDCA-3)-APC-Vio770	for 100 tests	130-110-261
CD141 (BDCA-3)-PerCP-Vio700	for 30 tests	130-110-363
CD141 (BDCA-3)-PerCP-Vio700	for 100 tests	130-110-262
CD141 (BDCA-3)-VioBright 515	for 30 tests	130-110-364
CD141 (BDCA-3)-VioBright 515	for 100 tests	130-110-263
CD141 (BDCA-3)-Biotin	for 30 tests	130-110-357
CD141 (BDCA-3)-Biotin	for 100 tests	130-110-256

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	CD141 (BDCA-3)
Clone	REA674
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	Thrombomodulin, Fetomodulin, TM
Entrez Gene ID	7056
Molecular mass of antigen [kDa]	59

Distribution of antigen	dendritic cells, monocytes, granulocytes
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA674 recognizes the human CD141 (BDCA-3) antigen, which is expressed at high levels on a minor subpopulation of human myeloid dendritic cells (about 0.02% of blood leukocytes). CD141 (BDCA-3)^{high} blood dendritic cells are CD11c^{dim}, CD123⁻, CD4⁺, Lin⁻, CD45RO⁺, CD2⁻, and CD16⁻. They express myeloid lineage markers, such as CD13 and CD33, and have a monocytoïd morphology. Unlike CD1c (BDCA-1)⁺ blood dendritic cells, CD141 (BDCA-3)^{high} blood dendritic cells lack expression of CD2 and Fc receptors such as CD32, CD64, or FcεRI. CD141 (BDCA-3) is also present at very low levels on CD14⁺ monocytes, granulocytes, CD303 (BDCA-2)⁺ CD304 (BDCA-4/Neuropilin-1)⁺ plasmacytoid, and CD1c (BDCA-1)⁺ myeloid dendritic cells. CD141 (BDCA-3)^{high} CD1c (BDCA-1)⁻ myeloid dendritic cells have been designated type-2 myeloid dendritic cells (MDC2s). CD141 is also known as thrombomodulin; thrombomodulin mediates co-agglutination by interaction with thrombin and protein C, though nothing is known about its function on MDC2s. Additional information: Clone REA674 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 Ca²⁺ or Mg²⁺ 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.

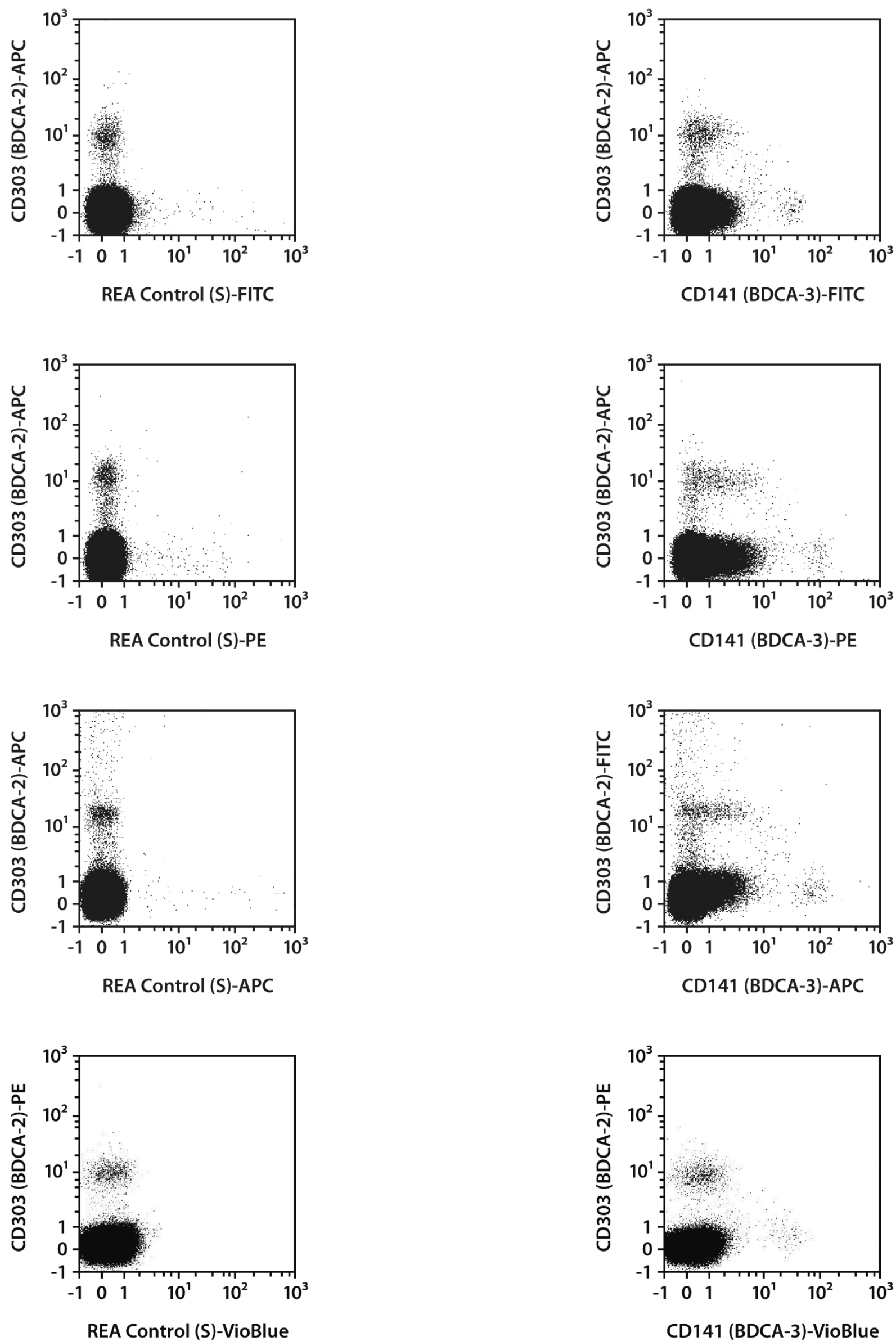
Protocol for cell surface staining

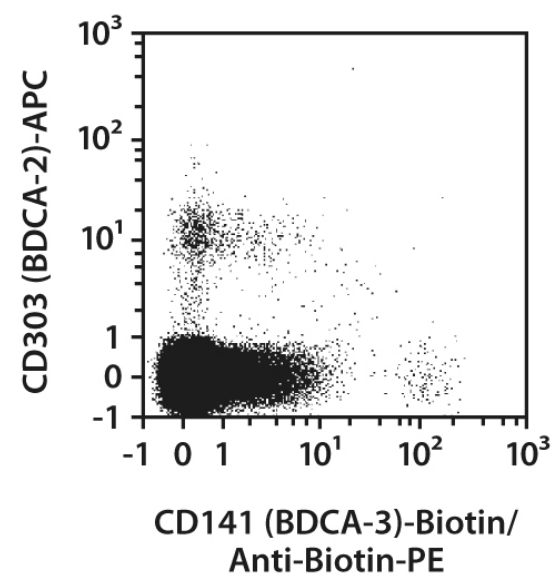
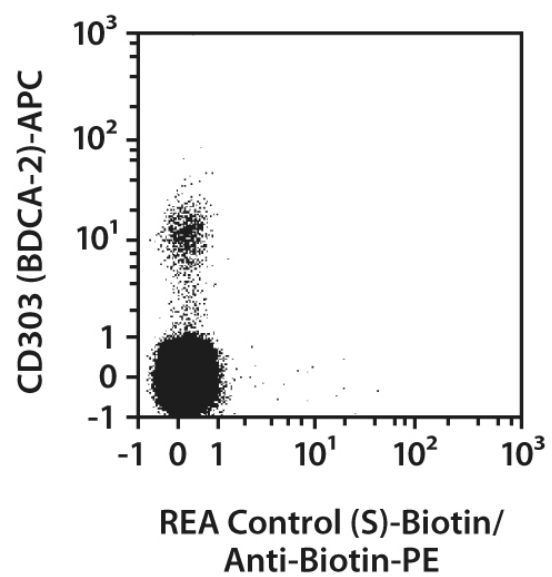
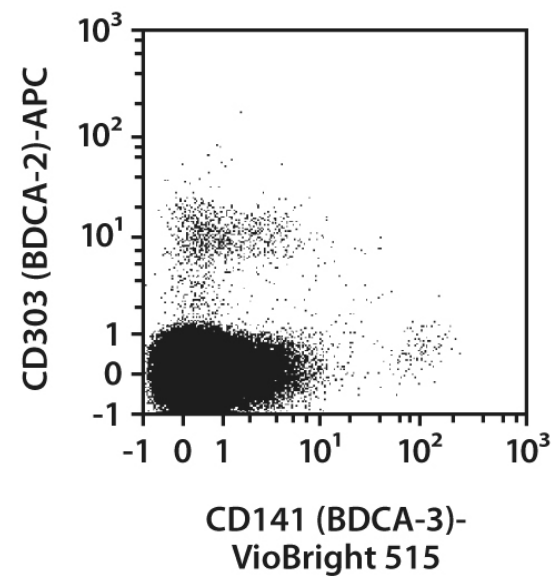
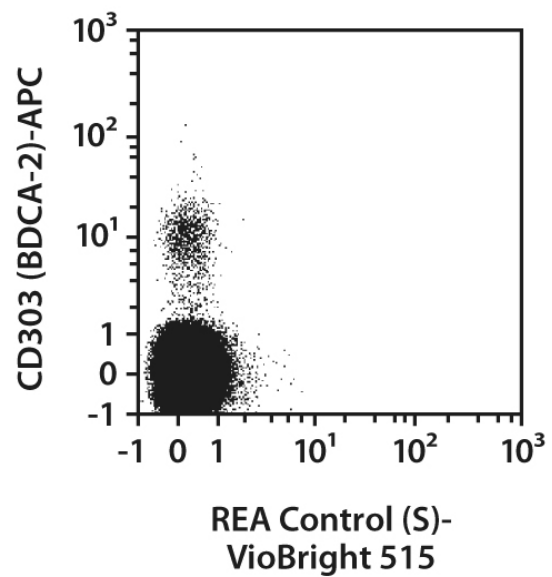
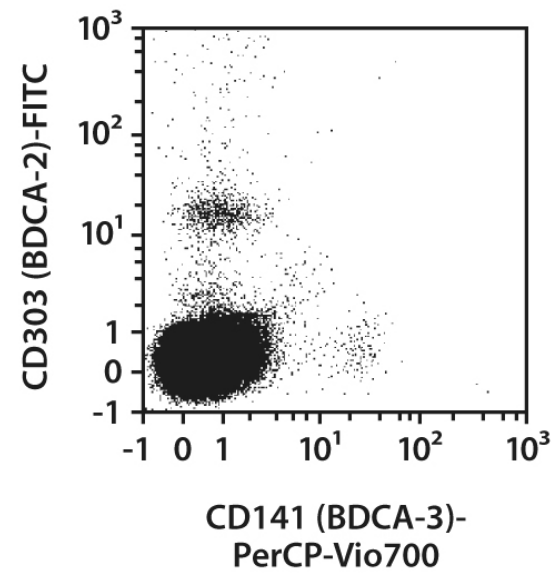
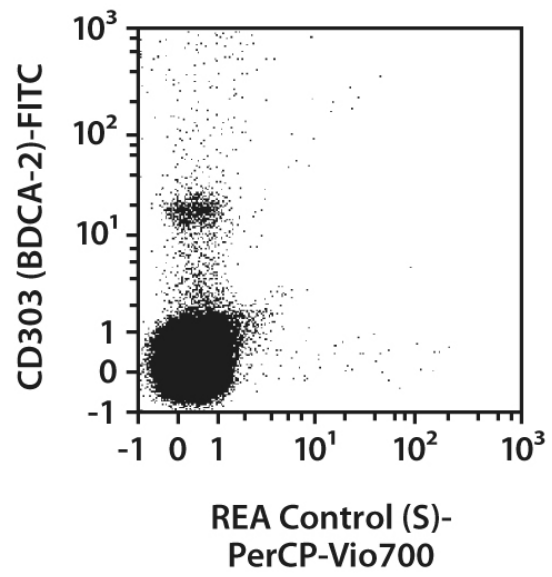
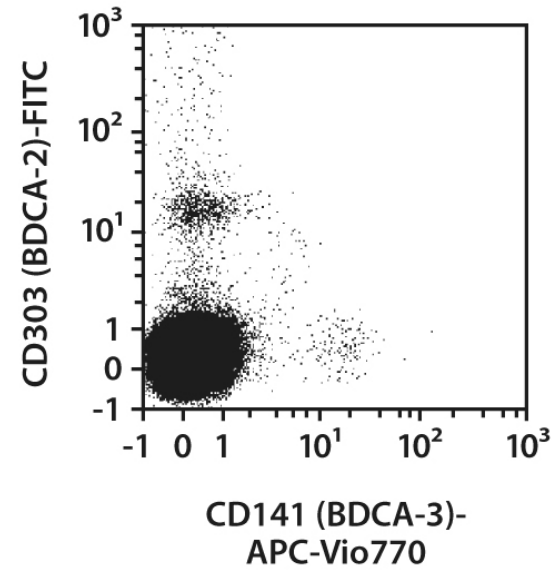
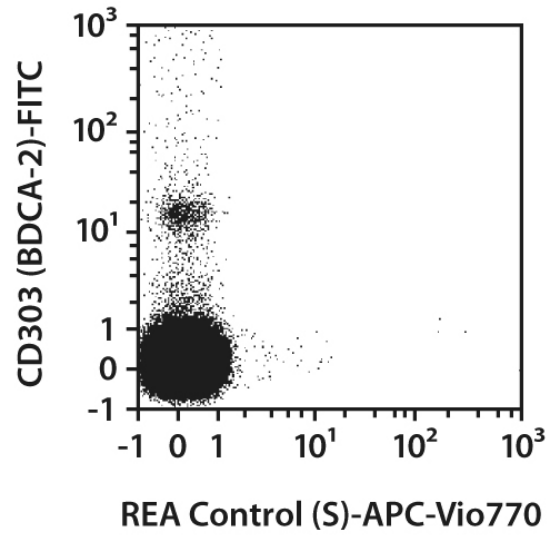
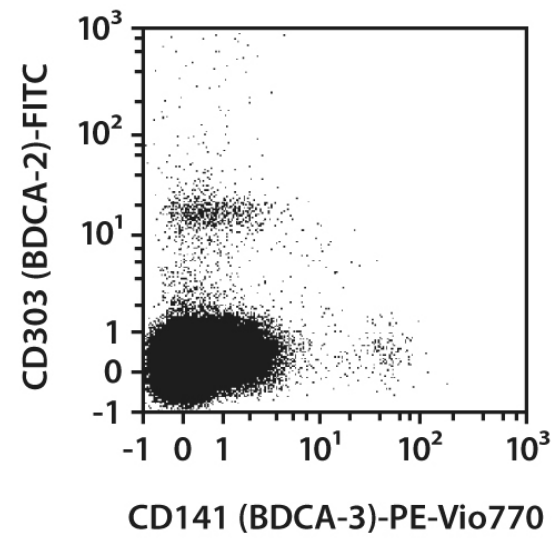
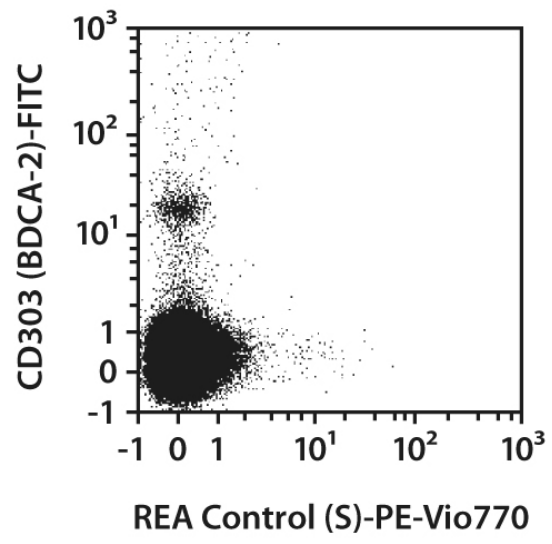
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10⁷ cells/100 µL of buffer.
- Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ 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 10⁷ 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 CD141 (BDCA-3) antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD303 (BDCA-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.





Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are either trademarks or registered trademarks of Miltenyi Biotec GmbH. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.