

CD141 (BDCA-3) antibodies, human

For research use only

One test corresponds to labeling of up to 10^6 cells in a total volume of 100 μ L

Product	Content	Order no.
CD141 (BDCA-3)-APC-Vio770	for 30 tests	130-113-877
CD141 (BDCA-3)-FITC	for 30 tests	130-113-879
CD141 (BDCA-3)-FITC	for 100 tests	130-113-317
CD141 (BDCA-3)-VioBright FITC	for 30 tests	130-113-883
CD141 (BDCA-3)-VioBright FITC	for 100 tests	130-113-321
CD141 (BDCA-3)-PE	for 30 tests	130-113-880
CD141 (BDCA-3)-PE	for 100 tests	130-113-318
CD141 (BDCA-3)-APC	for 30 tests	130-113-876
CD141 (BDCA-3)-APC	for 100 tests	130-113-314
CD141 (BDCA-3)-VioBlue	for 30 tests	130-113-882
CD141 (BDCA-3)-VioBlue	for 100 tests	130-113-320
CD141 (BDCA-3)-PE-Vio770	for 30 tests	130-113-881
CD141 (BDCA-3)-PE-Vio770	for 100 tests	130-113-319
CD141 (BDCA-3)-APC-Vio770	for 100 tests	130-113-315
CD141 (BDCA-3)-Biotin	for 30 tests	130-113-878
CD141 (BDCA-3)-Biotin	for 100 tests	130-113-316

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	AD5-14H12
Isotype	mouse IgG1k
Isotype control	Mouse IgG1 - isotype control antibodies
Alternative names of antigen	THBD, AHUS6, BDCA3, THPH12, THRM, TM
Entrez Gene ID	7056
Molecular mass of antigen [kDa]	59
Distribution of antigen	endothelial cells, megakaryocytes, monocytes, myeloid cells, neutrophils, platelets, smooth muscle

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 AD5-14H12 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.

Reagent requirements

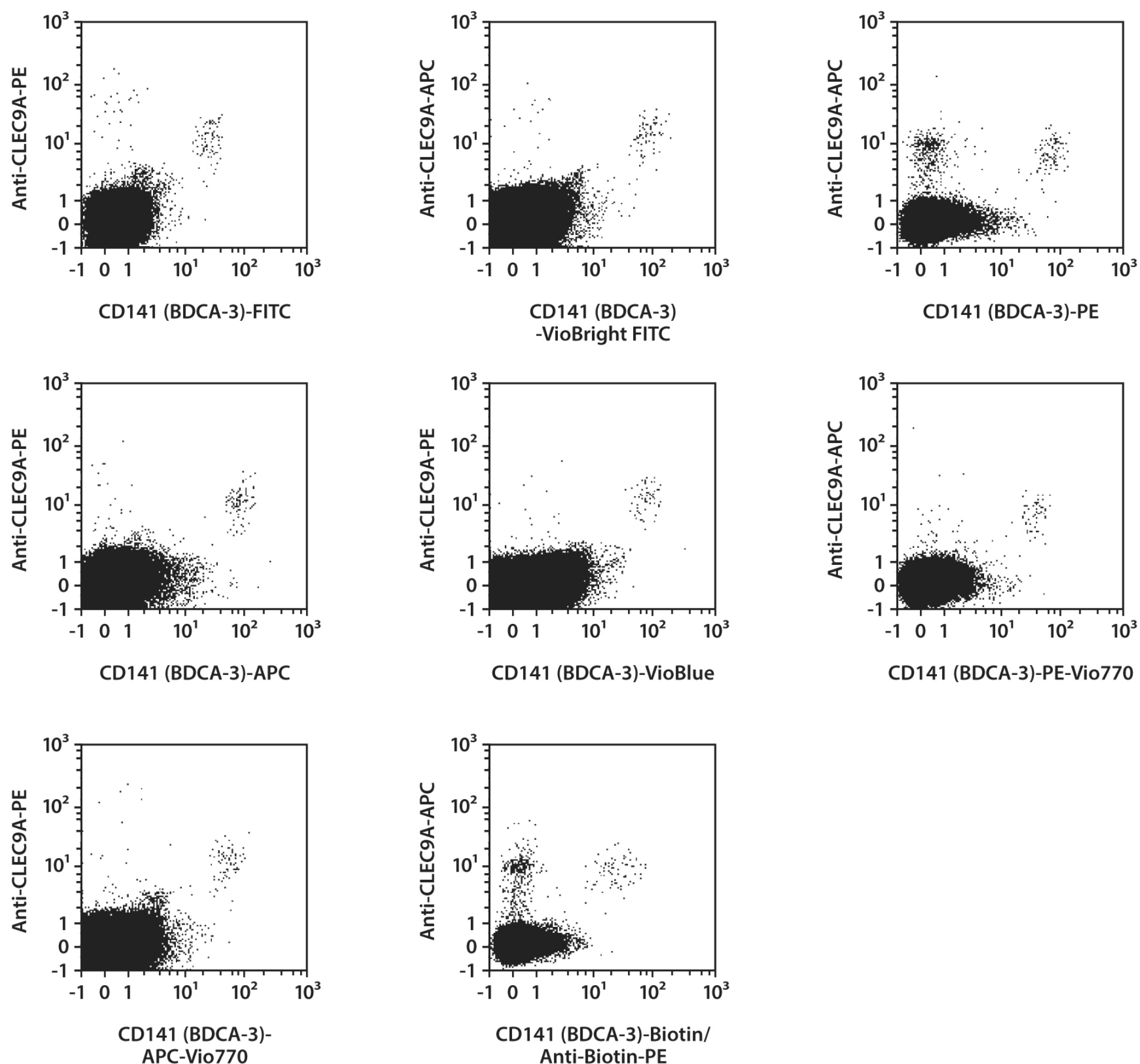
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other 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) 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 1:50 for up to 10⁶ cells/100 µL.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ 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 10⁶ 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 CD141 (BDCA-3) antibodies as well as with Anti-CLEC9A and CD45 antibodies and analyzed by flow cytometry using the MACSQuant®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. A pre-gate of CD45⁺ cells was used. 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

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