CD303 (BDCA-2) 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.
CD303 (BDCA-2)-FITC[1]	for 30 tests	130-097-927
CD303 (BDCA-2)-FITC[1]	for 100 tests	130-090-510
CD303 (BDCA-2)-PE[1]	for 30 tests	130-097-929
CD303 (BDCA-2)-PE[1]	for 100 tests	130-090-511
CD303 (BDCA-2)-APC[1]	for 30 tests	130-097-931
CD303 (BDCA-2)-APC[1]	for 100 tests	130-090-905
CD303 (BDCA-2)-VioBlue[1]	for 30 tests	130-106-505
CD303 (BDCA-2)-VioBlue[1]	for 100 tests	130-097-323
CD303 (BDCA-2)-PE-Vio615[1]	for 30 tests	130-107-508
CD303 (BDCA-2)-PE-Vio615[1]	for 100 tests	130-107-454
CD303 (BDCA-2)-PE-Vio770[1]	for 30 tests	130-106-392
CD303 (BDCA-2)-PE-Vio770[1]	for 100 tests	130-096-744
CD303 (BDCA-2)-Biotin[1]	for 30 tests	130-097-923
CD303 (BDCA-2)-Biotin[1]	for 100 tests	130-090-691
CD303 (BDCA-2) pure[1]	100 µg in 1 mL	130-090-690

[1]Not recommended for cells that are labeled with MACS MicroBeads using the same antigen.

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	CD303 (BDCA-2)
Clone	AC144
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	CLEC4C, BDCA-2, CLECSF11, CLECSF7, DLEC, HECL, PRO34150
Molecular mass of antigen [kDa]	25
Cross-reactivity	cynomolgus monkey (*Macaca fascicularis*)
Distribution of antigen	dendritic cells
Product format	Antibodies 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.

CD303 (BDCA-2) antibodies have been used, for example, to identify, characterize, and enumerate plasmacytoid dendritic cells in whole blood of healthy and HIV-infected individuals, and for analyzing the role of DC-SIGN in HIV infection and transmission.[7,11] Furthermore, CD303 (BDCA-2) antibodies were used to identify and enumerate plasmacytoid dendritic cells in blood and bone marrow samples before and after hematopoietic stem cell mobilization[8] or transplantation[10]. CD303 (BDCA-2) antibodies were also used for immunohistochemical staining, for example to identify plasmacytoid dendritic cells in tissue sections from patients with different inflammatory skin diseases.[5,12,13]

Clone AC144 recognizes the the CD303 (BDCA-2) antigen[1,3] which is expressed on human plasmacytoid dendritic cells in blood, lymphoid (e.g. tonsils and bone marrow) and non-lymphoid tissue.[3,6] Specific expression allows direct identification of plasmacytoid dendritic cells using just one marker.[1–11] CD303 (BDCA-2)$^{+}$ plasmacytoid dendritic cells in blood and bone marrow are CD11c^{-}, CD123^{high}, CD4^{+}, Lin^{-}, CD45RA^{+}, CD304 (BDCA-4/Neuropilin-1)$^{+}$, CD141 (BDCA-3)^{low}, CD1c (BDCA-1)$^{-}$, CD14^{-}, and CD2^{-}. They express neither myeloid lineage markers (CD13, CD33) nor Fc receptors (CD32, CD64, FcRI).[1,6] CD303 (BDCA-2) is strongly expressed on freshly isolated plasmacytoid dendritic cells but down-regulated within 48 hours of culturing.[1] Unlike CD304 (BDCA-4/Neuropilin-1), CD303 (BDCA-2) is not detectable on *ex vivo* generated monocyte-derived or hematopoietic precursor cell-derived CD1a^{+} dendritic cells.[1,9]

The CD303 (BDCA-2) antigen is a novel type II transmembrane C-type lectin.[3] Remarkably, plasmacytoid dendritic cells can take up ligands via CD303 (BDCA-2), then process and present the ligands to T cells.[1,3] Unlike binding of antibodies to CD304 (BDCA-4), binding of antibodies to CD303 (BDCA-2) inhibits type I IFN production, which is induced in plasmacytoid dendritic cells by, for example, the influenza virus.[3,6]

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) 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

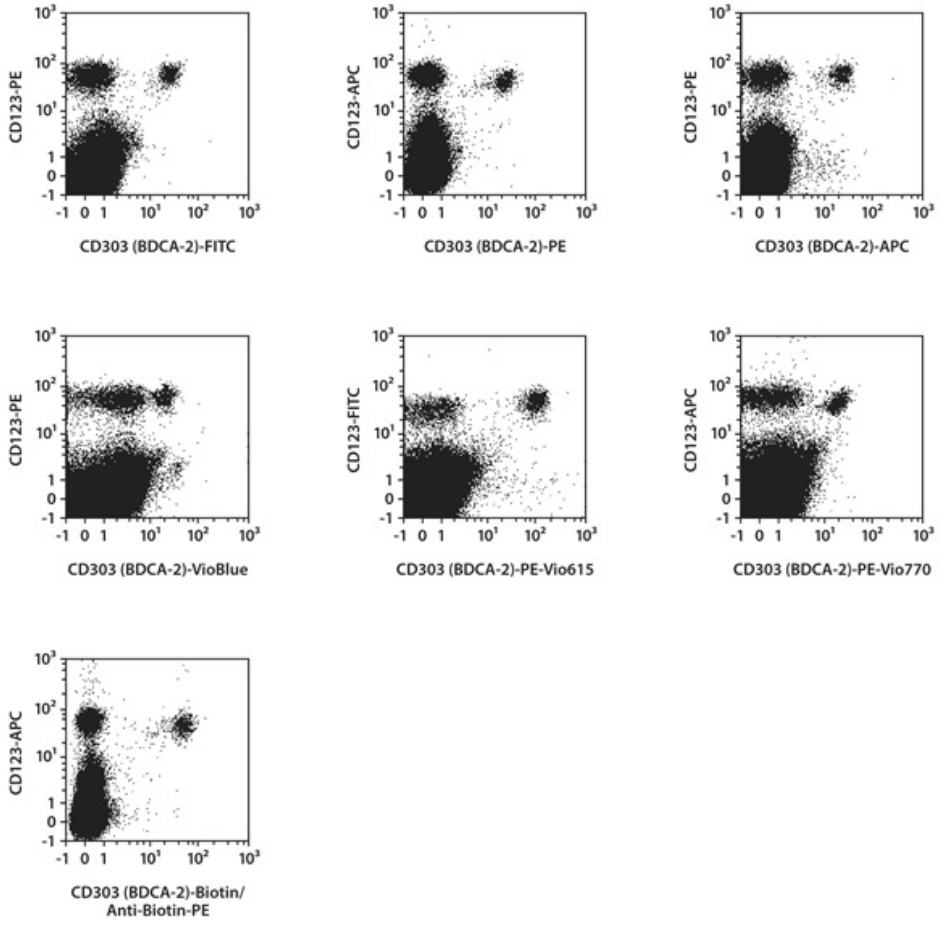
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher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

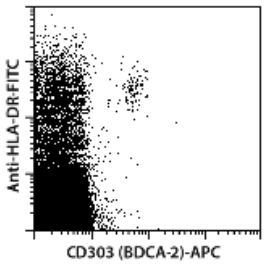
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased 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 CD303 (BDCA-2), CD123 and CD45 antibodies and analyzed by flow cytometry. 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 pregate 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 tandems.



PBMCs from cynomolgus monkey (*Macaca fascicularis*) were stained with CD303 (BDCA-2)-APC and Anti-HLA-DR-FITC and analyzed by flow cytometry.



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