

## Contents

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## 1. Description

<b>Components</b>	1 mL monoclonal CD141 (BDCA-3) antibodies, human conjugated to various dyes.												
	<table border="0"> <tr> <td>FITC</td> <td>130-090-513</td> </tr> <tr> <td>PE</td> <td>130-090-514</td> </tr> <tr> <td>APC</td> <td>130-090-907</td> </tr> <tr> <td>VioBlue®</td> <td>130-097-325</td> </tr> <tr> <td>Biotin</td> <td>130-090-749</td> </tr> <tr> <td>pure</td> <td>130-090-694</td> </tr> </table>	FITC	130-090-513	PE	130-090-514	APC	130-090-907	VioBlue®	130-097-325	Biotin	130-090-749	pure	130-090-694
FITC	130-090-513												
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VioBlue®	130-097-325												
Biotin	130-090-749												
pure	130-090-694												
<b>Clone</b>	AD5-14H12 (isotype: mouse IgG1).												
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.												
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.												
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.												

### 1.1 Background information

The CD141 (BDCA-3) antigen is expressed at high levels on a minor subpopulation of human myeloid dendritic cells in blood (about 0.02% of blood leukocytes).<sup>1</sup> CD141 (BDCA-3)<sup>++</sup> blood dendritic cells are CD1c (BDCA-1)<sup>-</sup>, CD11c<sup>dim</sup>, CD123<sup>-</sup>, CD4<sup>+</sup>, Lin<sup>-</sup>, CD45RO<sup>+</sup>, CD2<sup>-</sup>, and CD16<sup>-</sup>. They express myeloid lineage markers, such as CD13 and CD33, and have a monocytoïd morphology. Unlike CD1c (BDCA-1)<sup>+</sup> blood dendritic cells, CD141 (BDCA-3)<sup>++</sup> blood dendritic cells lack expression of CD2 and Fc receptors like CD32, CD64, or FcεR1.<sup>1</sup> CD141 (BDCA-3) is also present at very low levels on CD14<sup>+</sup> monocytes, granulocytes, CD303 (BDCA-2)<sup>+</sup> plasmacytoïd and CD1c (BDCA-1)<sup>+</sup> myeloid blood dendritic cells. CD141 (BDCA-3)<sup>++</sup> CD1c (BDCA-1)<sup>-</sup> myeloid dendritic cells have been designated type-2 myeloid dendritic cells (MDC2s). CD141 (Thrombomodulin) was described to mediate coagulation by interaction with thrombin and protein C. However, nothing is known about its function on MDC2s.

### 1.2 Applications

- Identification and enumeration of CD141 (BDCA-3)<sup>++</sup> myeloid dendritic cells in peripheral blood or lymphoid and non-lymphoid tissue by immunofluorescent or immunocytochemical staining and flow cytometric or microscopic analysis. The antibodies are suitable for staining of fresh or formaldehyde-fixed cells in suspension and for staining of e.g. air-dried, acetone-fixed cryosections. They are not suitable for paraffin-embedded tissue sections.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human myeloid dendritic cells can be isolated by using, for example, CD141 (BDCA-3) MicroBead Kit, human (# 130-090-512).
- CD141 (BDCA-3) antibodies were used for, e.g. identification, enumeration, and characterization of CD141 (BDCA-3)<sup>++</sup> myeloid dendritic cells in peripheral blood mononuclear cells (PBMCs)<sup>1-3</sup> or in whole blood of healthy subjects and HIV-infected individuals<sup>4</sup>, or their localization in cryosections of lymphoid and non-lymphoid tissue by immunohistochemical staining<sup>3</sup>.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD141 (BDCA-3) conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD141 (BDCA-3) MicroBead-labeled cells use the same dilution.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

### 1.4 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). 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) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD141 (BDCA-3)-Biotin.
- (Optional) CD14-FITC (# 130-080-701) or CD1c (BDCA-1)-PE (# 130-090-508). For more information about antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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.

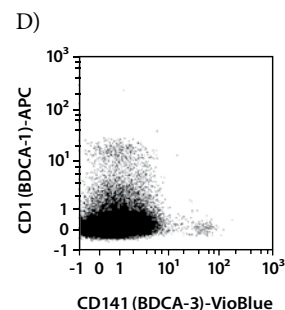
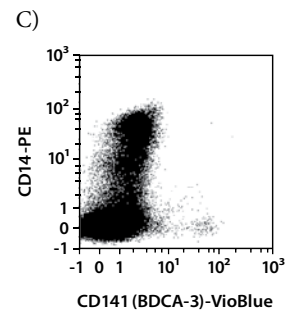
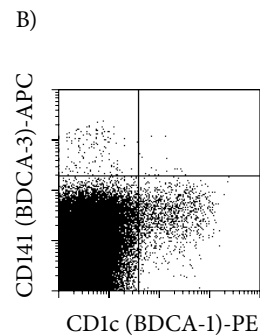
## 2. General protocol for immunofluorescent staining

▲ 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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 10  $\mu$ L of the CD141 (BDCA-3) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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 CD141 (BDCA-3)-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of 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.

## 3. Examples of immunofluorescent staining with CD141 (BDCA-3) antibodies

PBMCs were stained with CD141 (BDCA-3)-APC or CD141 (BDCA-3)-VioBlue, CD14-FITC (# 130-080-701) or CD14-PE (# 130-091-242), CD1c (BDCA-1)-PE (# 130-090-508) or CD1c (BDCA-1)-APC (# 130-090-903) and CD19-Cy<sup>™</sup>-Chrome. Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence. For discrimination of CD141 (BDCA-3)<sup>++</sup> and CD1c (BDCA-1)<sup>+</sup> myeloid dendritic cells, B cells were excluded from the analysis based on CD19 expression (B), (D).



For more examples please refer to the respective product page at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

#### 4. References

1. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3, BDCA-4: Three marker for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* 165: 6037–6046.
2. MacDonald, K. P. A. *et al.* (2002) Characterization of human blood dendritic cell subsets. *Blood* 100: 4512–4520.
3. Lebre, C. *et al.* (2003) BDCA-3<sup>hi</sup> dendritic cells: a novel subset with distinct phenotypical characteristics. Doctoral dissertation, University of Amsterdam, Netherlands.
4. Chehimi, J. *et al.* (2002) Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J. Immunol.* 168: 4769–4801.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

#### 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.

#### Warranty

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