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### 1. Description

<b>Components</b>	1 mL monoclonal CD11c antibodies, human conjugated to various dyes.												
	<table border="0"> <tr> <td>VioBlue®</td> <td>130-097-328</td> </tr> <tr> <td>FITC</td> <td>130-092-410</td> </tr> <tr> <td>PE</td> <td>130-092-411</td> </tr> <tr> <td>APC</td> <td>130-092-412</td> </tr> <tr> <td>Biotin</td> <td>130-092-413</td> </tr> <tr> <td>pure</td> <td>130-092-414</td> </tr> </table>	VioBlue®	130-097-328	FITC	130-092-410	PE	130-092-411	APC	130-092-412	Biotin	130-092-413	pure	130-092-414
VioBlue®	130-097-328												
FITC	130-092-410												
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APC	130-092-412												
Biotin	130-092-413												
pure	130-092-414												
<b>Clone</b>	MJ4-27G12.4.6 (isotype: mouse IgG2b).												
<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

CD11c is a 145–150 kDa type I transmembrane glycoprotein also known as integrin  $\alpha$ x and CR4. CD11c non-covalently associates with  $\beta$ 2 integrin (CD18) to form a CD11c/CD18 heterodimer and is expressed on monocytes, macrophages, myeloid dendritic cells (MDCs), granulocytes, NK cells<sup>1</sup>, and subsets of T and B cells. On myeloid dendritic cells, CD11c is highly expressed on type 1 myeloid dendritic cells (CD1c (BDCA-1)<sup>+</sup>, CD123<sup>low</sup> MDC1s) and at low levels on type 2 myeloid dendritic cells (CD141 (BDCA-3)<sup>+</sup>, CD1c (BDCA-1)<sup>-</sup>, CD123<sup>-</sup> MDC2s).<sup>2,3</sup>

CD11c has been reported to play a role in adhesion and CTL killing through its interactions with fibrinogen, CD54, and iC3b.

#### 1.2 Applications

- Identification and enumeration of CD11c<sup>+</sup> cells by flow cytometry or fluorescence microscopy.

- Evaluation of MACS® separations by flow cytometry or fluorescence microscopy. Human monocytes can be isolated by using e.g. CD14 MicroBeads, human (# 130-050-201). Myeloid dendritic cells can be isolated by using the CD1c (BDCA-1)<sup>+</sup> Dendritic Cell Isolation Kit, human (# 130-090-506).

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD11c 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.

The antibody is suited for staining of formaldehyd-fixed cells.

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., APC (# 130-090-856) as secondary antibody reagent in combination with CD11c-Biotin.
- (Optional) Mouse IgG2b isotype control antibodies conjugated to, e.g., PE (# 130-092-245). 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

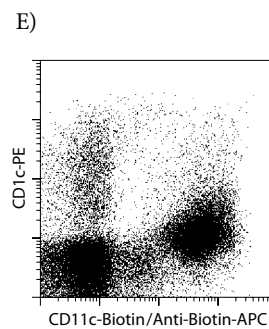
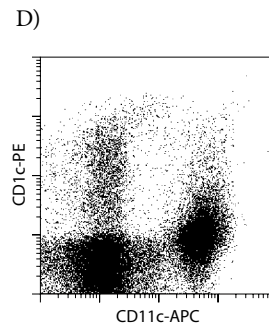
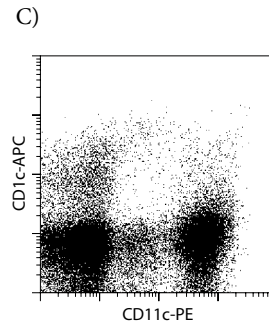
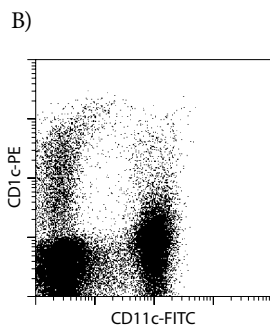
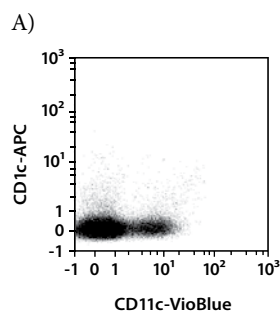
▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10<sup>7</sup> nucleated cells, use twice 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<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CDxx 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 CD11c-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL 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 CD11c antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD11c antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) as well as with CD1c (BDCA-1)-APC (# 130-090-903) or CD1c (BDCA-1)-PE (# 130-090-508) and analyzed by flow cytometry. Cells labeled with CD11c-Biotin (E) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD1c (BDCA-1)-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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

### 4. References

1. Barclay, A.N. *et al.* (1997) The Leukocyte Antigen Facts Book. (2nd Edition) Academic Press, San Diego, CA, pp. 161–162.
2. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* 165: 6037–6046.
3. Bendiss-Vermare, N. (2001) Human thymus contains IFN-alpha-producing CD11c(-), myeloid CD11c(+), and mature interdigitating dendritic cells. *J. Clin. Invest.* 107: 835–844.

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**

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.

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