

# CD11c antibodies, human

For research use only

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

Product	Content	Order no.
CD11c-VioBright FITC	for 30 tests	130-109-676
CD11c-VioBright FITC	for 100 tests	130-109-617
CD11c-PE	for 30 tests	130-109-672
CD11c-PE	for 100 tests	130-109-613
CD11c-PE-Vio615	for 30 tests	130-111-937
CD11c-PE-Vio615	for 100 tests	130-111-775
CD11c-PE-Vio770	for 30 tests	130-109-674
CD11c-PE-Vio770	for 100 tests	130-109-615
CD11c-APC-Vio770	for 30 tests	130-109-675
CD11c-APC-Vio770	for 100 tests	130-109-616
CD11c-Biotin	for 30 tests	130-109-671
CD11c-Biotin	for 100 tests	130-109-612

## 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

<b>Antigen</b>	CD11c
<b>Clone</b>	REA618
<b>Isotype</b>	recombinant human IgG1
<b>Isotype control</b>	REA Control (S) antibodies
<b>Alternative names of antigen</b>	95, CR4, integrin $\alpha$ X, ITGAX, p150
<b>Molecular mass of antigen [kDa]</b>	126
<b>Distribution of antigen</b>	B cells, dendritic cells, granulocytes, leukemia cells, lymphocytes, macrophages, monocytes, myeloid cells, NK cells, T cells
<b>Product format</b>	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Fixation</b>	The antibody is suited for staining of formaldehyde-fixed cells.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze.

Clone REA618 recognizes the human CD11c antigen, a 145–150 kDa type I transmembrane

glycoprotein, which is also known as integrin  $\alpha$ X or CR4. It is expressed on monocytes, macrophages, NK cells, granulocytes, myeloid dendritic cells (MDCs), 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 low on type 2 myeloid dendritic cells (CD1c (BDCA-1)<sup>-</sup> CD123<sup>-</sup> MDC2s). CD11c, also known as integrin  $\alpha$ X or CR4, has been reported to play a role in adhesion and CTL killing through its interactions with fibrinogen, CD54, and iC3b.  
Additional information: Clone REA618 displays negligible binding to Fc receptors.

## Reagent requirements

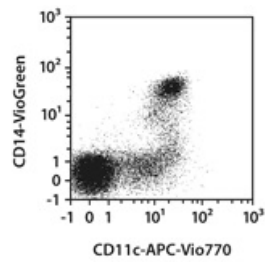
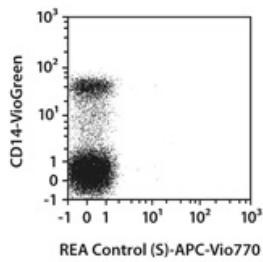
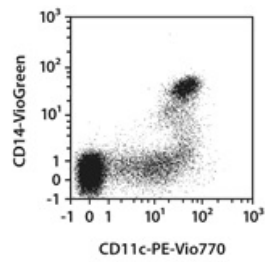
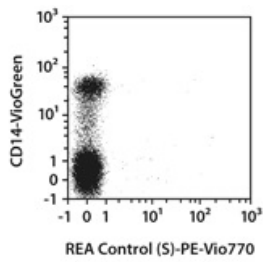
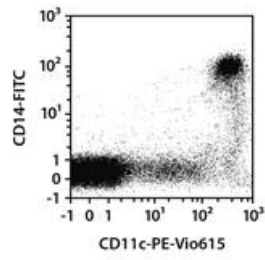
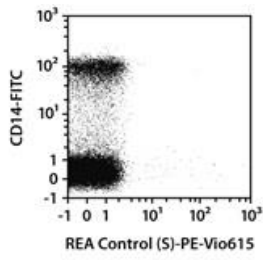
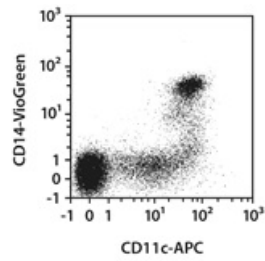
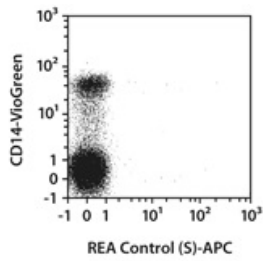
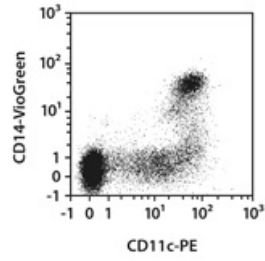
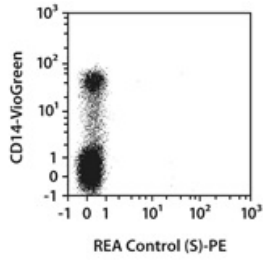
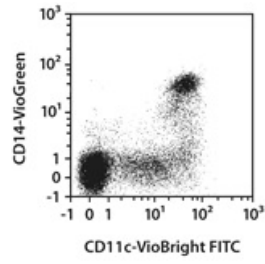
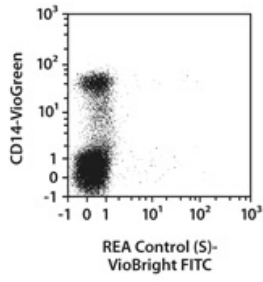
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> 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.

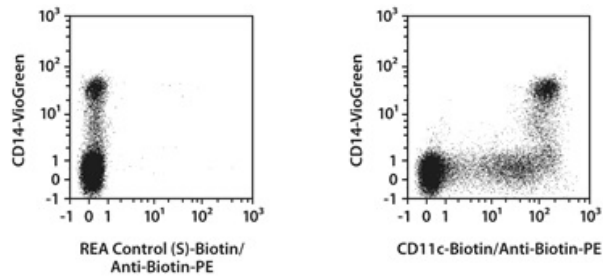
## Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10<sup>7</sup> cells/100  $\mu$ L of buffer.
  - 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 $\times$ 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 $\times$ g for 10 minutes. Aspirate supernatant completely.
  3. Resuspend up to 10<sup>7</sup> nucleated cells per 100  $\mu$ L of buffer.
  4. Add 10  $\mu$ L 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 $\times$ g for 10 minutes. Aspirate supernatant completely.
  7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L 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 CD11c antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD14 antibodies. Flow cytometry was performed using the MACSQuant<sup>®</sup> 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.





## References

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

## Warranty

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**Miltenyi Biotec GmbH** | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | [macs@miltenyibiotec.de](mailto:macs@miltenyibiotec.de) | [www.miltenyibiotec.com](http://www.miltenyibiotec.com)  
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