CD11b antibodies
human and mouse

Miltenyi Biotec

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1. Description

Components	1 mL monoclonal CD11b antibodies, human and mouse conjugated to various dyes.
	FITC 130-081-201 PE 130-091-240 APC 130-091-241 VioBlue® 130-097-336 VioGreen™ 130-097-299 APC-Vio770™ 130-096-834
Clone	M1/70.15.11.5 (isotype: rat IgG2b).
Capacity	100 tests or up to 10^9 total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

Cross-reactivity: The CD11b antibody has been reported to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

1.1 Background information

The CD11b (Mac-1 α; integrin αM chain) antibody reacts with the 170 kDa αM subunit of CD11b/CD18 heterodimer (Mac-1, $\alpha_M\beta_2$ intergrin). It functions as a receptor for complement (C3bi), fibrinogen or clotting factor X.
The monoclonal CD11b antibody recognizes the human, mouse and non-human primate CD11b antigen. In humans, CD11b is strongly expressed on myeloid cells and microglia, and weakly expressed on NK cells and some activated lymphocytes. In mouse, the CD11b antigen is expressed on monocytes/macrophages and microglia[1-3], and to a lower extent on granulocytes, NK cells, $CD5^+$ B1 cells, and a subset of dendritic cells. In rhesus monkey, CD11b is expressed on myeloid cells, including monocytes and granulocytes.

1.2 Applications

- Identification and enumeration of $CD11b^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human or mouse monocytes can be isolated by using, for example, CD11b MicroBeads, human or mouse (# 130-049-601). Human or mouse microglia can be isolated using CD11b (Microglia) MicroBeads (# 130-093-634).[1-3] Rhesus monkey (*Macaca mulatta*) $CD11b^+$ cells can be isolated by using CD11b MicroBeads, non-human primate (# 130-091-100).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD11b conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry (for microglia cells: 10^6 cells/100 µL of buffer).
For optimal results, cells must be stained prior to fixation with formaldehyde.

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^{2+} or Mg^{2+} are not recommended for use.
- (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 2×10^7 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.

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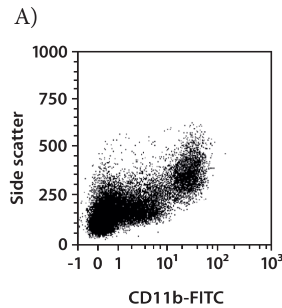
4. Add 10 µL of the CD11b antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

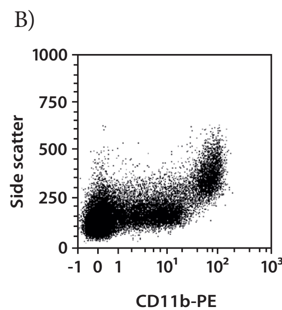
3. Examples of immunofluorescent staining with CD11b antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD11b antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), or APC-Vio770 (F) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

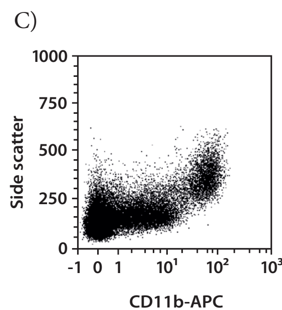
A)



B)



C)



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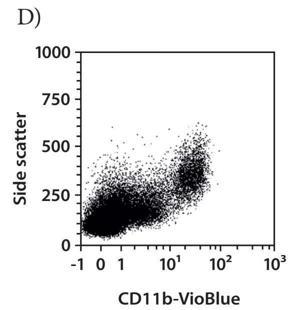

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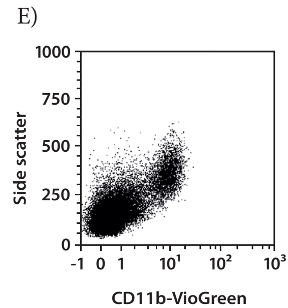

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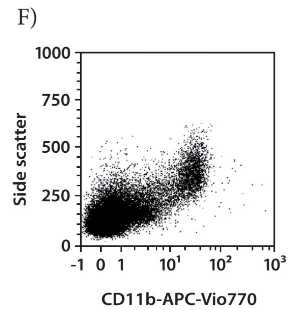
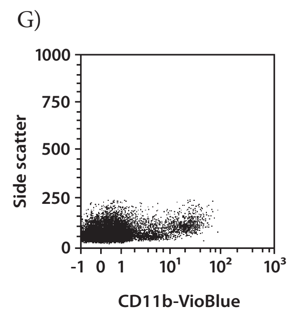


Mouse spleen cells were stained with CD11b-VioBlue (G) and CD11b-PE (J) and rhesus monkey PBMCs (H) were stained with CD11b-PE and analyzed by flow cytometry. Mouse brain cells were stained with CD11b-APC (I) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

G)



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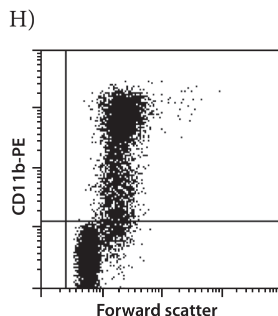
H)



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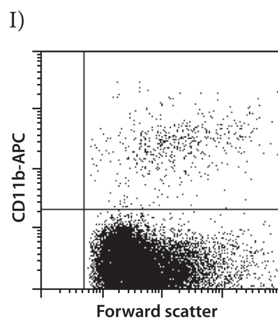
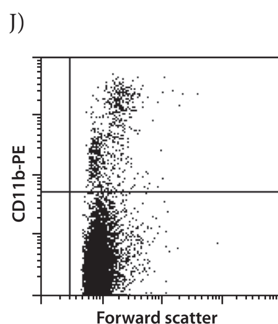
I)



J)



4. References

1. Boddaert, J. *et al.* (2007) Evidence of a role for lactadherin in Alzheimer's disease. Am. J. Pathol. 170: 921–929.
2. Li, H. *et al.* (2006) Different neurotropic pathogens elicit neurotoxic CCR9- or neurosupportive CXCR3-expressing microglia. J. Immunol. 177: 3644–3656.
3. Ndhlovu, L. C. *et al.* (2001) Critical involvement of OX40 ligand signals in the T cell priming events during experimental autoimmune encephalomyelitis. J. Immunol. 167: 2991–2999.

All protocols and data sheets are available at **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.

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