CD11b/c antibodies, rat

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD11b/c-FITC	9 µg in 300 µL	130-105-315
CD11b/c-FITC	30 µg in 1 mL	130-105-273
CD11b/c-PE	9 µg in 300 µL	130-105-316
CD11b/c-PE	30 µg in 1 mL	130-105-274
CD11b/c-APC	9 µg in 300 µL	130-105-317
CD11b/c-APC	30 µg in 1 mL	130-105-275
CD11b/c-PE-Vio770	9 µg in 300 µL	130-105-318
CD11b/c-PE-Vio770	30 µg in 1 mL	130-105-276
CD11b/c-APC-Vio770	9 µg in 300 µL	130-105-319
CD11b/c-APC-Vio770	30 µg in 1 mL	130-105-277
CD11b/c-Biotin	9 µg in 300 µL	130-105-314
CD11b/c-Biotin	30 µg in 1 mL	130-105-272
CD11b/c pure	100 µg in 1 mL	130-105-127

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	CD11b/c
Clone	REA325
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	ITGAM, ITGAX, Leukocyte adhesion glycoprotein p150,95 α chain (Leukocyte adhesion receptor p150,95), RGD1561123, CR-3 α chain, Cell surface glycoprotein MAC-1 subunit α, Leukocyte adhesion receptor MO1, Neutrophil adherence receptor, CD11b
Molecular mass of antigen [kDa]	128
Distribution of antigen	monocytes, granulocytes, macrophages, dendritic cells, NK cells, lymphocytes
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.

Clone REA325 recognizes the rat CD11b and c antigen, which are both Single-pass type I membrane glycoproteins also known as Integrin α-M (CD11b) and Integrin α-X (CD11c). CD11c and CD11b are expressed on monocytes, granulocytes, macrophages, dendritic cells, NK cells, and a subset of lymphocytes.
Additional information: Clone REA325 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® 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) 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.

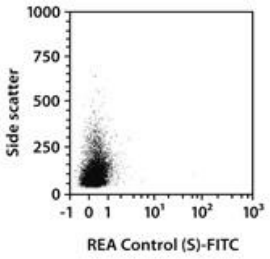
Protocol for cell surface staining

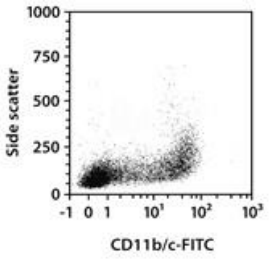
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

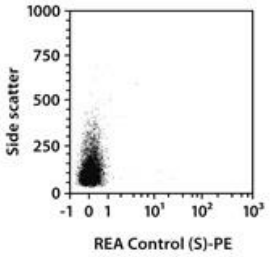
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

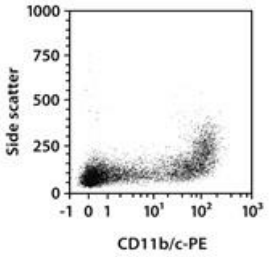
Examples of immunofluorescent staining

Splenocytes from Lewis rats were stained with CD11b/c antibodies conjugated to PE or with the corresponding REA control (S) antibodies (left image) 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.


Side scatter
REA Control (S)-FITC


Side scatter
CD11b/c-FITC


Side scatter
REA Control (S)-PE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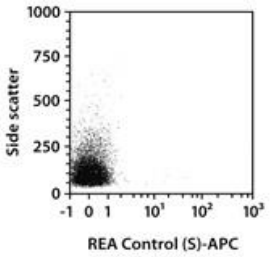
CD11b/c-PE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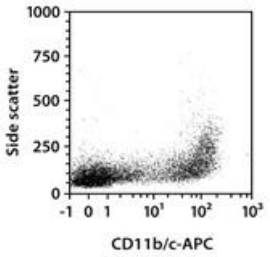
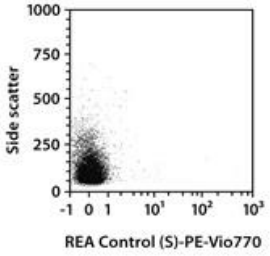
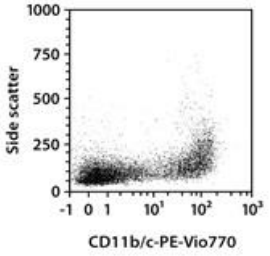
REA Control (S)-APC


Side scatter
CD11b/c-APC


Side scatter
REA Control (S)-PE-Vio770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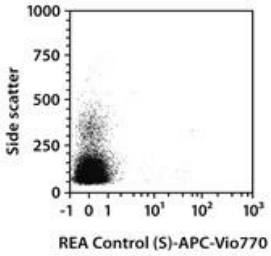
CD11b/c-PE-Vio770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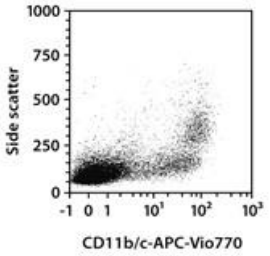
REA Control (S)-APC-Vio770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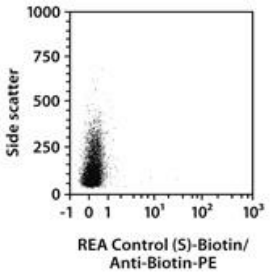
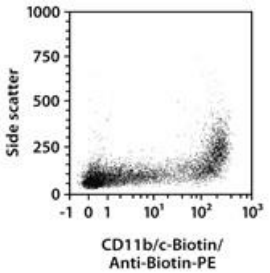
CD11b/c-APC-Vio770


Side scatter
REA Control (S)-Biotin/
Anti-Biotin-PE


Side scatter
CD11b/c-Biotin/
Anti-Biotin-PE

References

1. **Hubert, F. X. *et al.*** (2004) Rat plasmacytoid dendritic cells are an abundant subset of MHC Class II^{+} CD4^{+}CD11b^{-}OX62^{-} and type I IFN-producing cells that exhibit selective expression of Toll-like receptors 7 and 9 and strong responsiveness to CpG. J. Immunol. 172(12): 7485–7494.
2. **Kriegel, M. A. *et al.*** (2012) Pancreatic islet expression of chemokine CCL2 suppresses autoimmune diabetes via tolerogenic CD11c+ CD11b+ dendritic cells. Proc. Natl. Acad. Sci. U.S.A. 109(9): 3457–3462.
3. **Milling, S. *et al.*** (2010) Subsets of migrating intestinal dendritic cells. Immunol. Rev. 234(1): 259–267.

Warranty

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