CD49b antibodies, mouse

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.
CD49b-PE	9 µg in 300 µL	130-108-200
CD49b-PE	30 µg in 1 mL	130-108-174
CD49b-APC	9 µg in 300 µL	130-108-201
CD49b-APC	30 µg in 1 mL	130-108-175
CD49b-PE-Vio770	9 µg in 300 µL	130-108-202
CD49b-PE-Vio770	30 µg in 1 mL	130-108-176
CD49b-APC-Vio770	9 µg in 300 µL	130-108-203
CD49b-APC-Vio770	30 µg in 1 mL	130-108-177
CD49b-Biotin	9 µg in 300 µL	130-108-199
CD49b-Biotin	30 µg in 1 mL	130-108-173

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	CD49b
Clone	REA541
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	Itga2, gpIa, VLA-2α, VLA-2alpha, DX5
Molecular mass of antigen [kDa]	126
Distribution of antigen	NK cells, T helper cells, NKT cells
Product format	Reagents 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 REA541 recognizes the mouse CD49b antigen, a single-pass type I membrane protein also known as integrin alpha-2 (ITGA2). CD49b together with CD29 (ITGB1) forms the adhesion molecule very late antigen-2 (VLA-2), which binds mainly to collagens I, II, and XI. VLA-2 is responsible for adhesion of platelets and other cells to collagens, modulation of collagen and collagenase gene expression, force generation, and organization of newly synthesized extracellular matrix. It is also a

receptor for laminins, collagen C-propeptides, and E-cadherin. Mice homozygous for a null mutation in the CD49b gene die very early in embryogenesis. CD49b is expressed on the vast majority of mouse NK cells and on a subset of NKT cells. It is not as mouse strain-restricted as other NK cell markers, for example, NK1.1, and is expressed by most common inbred mouse strains. Bone marrow (BM) resident memory T helper (TH) cells also express CD49b and the homing of adoptively transferred BM memory TH cells to BM can be efficiently blocked by antibodies to CD49b. This points to a decisive role of CD49b in the trafficking of memory Th cell precursors to the BM niches for memory TH cells.
Additional information: Clone REA541 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.

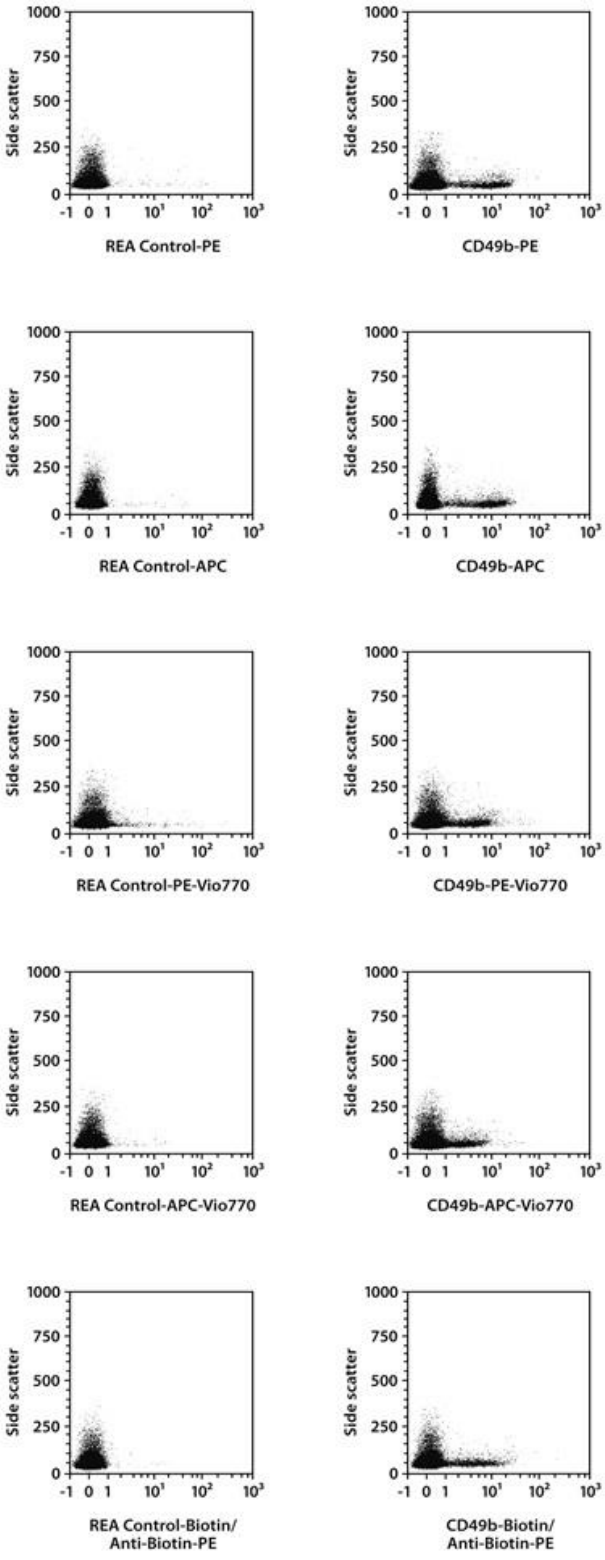
Protocol for cell surface staining

- 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 BALB/c mice were stained with CD49b antibodies or with the corresponding REA Control antibodies (left image). Flow cytometry was performed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Edelman, J. M. *et al.*** (1994) The mouse VLA-2 homologue supports collagen and laminin adhesion but not virus binding. Cell Adhes. Commun. 2(2): 131–143.
2. **Gagliani, N. *et al.*** (2013) Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nat. Med. 19(6): 739–746.
3. **Hanazawa, A. *et al.*** (2013) CD49b/CD69-dependent generation of resting T helper cell memory. Front Immunol 4: 183–183.

Warranty

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