CD49d antibodies, mouse

For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10 cells.

Product	Content	Order no.
CD49d-Biotin	30 µg in 200 µL	130-111-976
CD49d-PE	30 µg in 200 µL	130-111-977
CD49d-PE	150 µg in 1 mL	130-111-824
CD49d-APC	30 µg in 200 µL	130-111-978
CD49d-APC	150 µg in 1 mL	130-111-825
CD49d-PE-Vio770	30 µg in 200 µL	130-111-979
CD49d-PE-Vio770	150 µg in 1 mL	130-111-826
CD49d-Biotin	150 µg in 1 mL	130-111-823

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	CD49d
Clone	REA807
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	ITGA4, Itga4B, VLA-4α, Integrin alpha-4
Entrez Gene ID	16401
Molecular mass of antigen [kDa]	111
Distribution of antigen	B cells, basophils, cardiac muscle, dendritic cells, eosinophils, Langerhans cells, lymphocytes, macrophages, mast cells, monocytes, myeloid cells, NK cells, placenta, red blood cells, skeletal muscle, smooth muscle, T cells, thymocytes
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA807 recognizes the mouse CD49d antigen, a 150 kDa integrin family member, also known as integrin α4 chain. Integrins are cell-surface receptors, expressed as heterodimers essential in various processes mediating intercellular or cell-matrix interaction. CD49d associates non-covalently with the integrin ß or ß1 (CD29) forming the complex VLA4, which is expressed on peripheral lymphocytes, thymocytes, eosinophils, basophils, mast cells, dendritic cells, and monocytes. VLA4 plays a critical role in adhesion and migration of cells into tissues by binding to its ligand VCAM-1 (CD106). Together with the ß7 integrin, CD49d forms the heterodimer LPAM-1, which is a ligand for the mucosal vascular addressin (MAdCAM-1).

Additional information: Clone REA807 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.

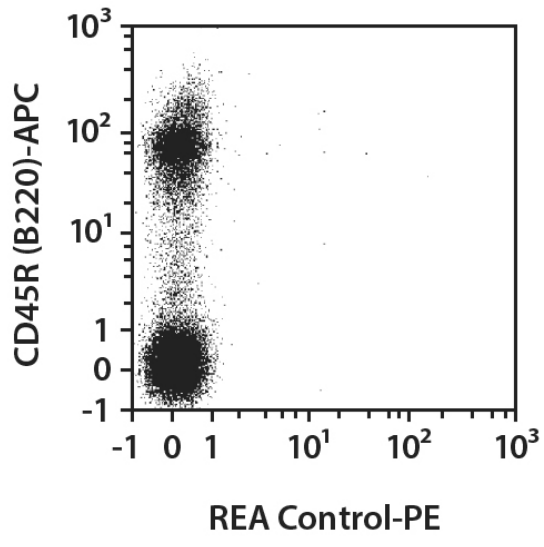
Protocol for cell surface staining

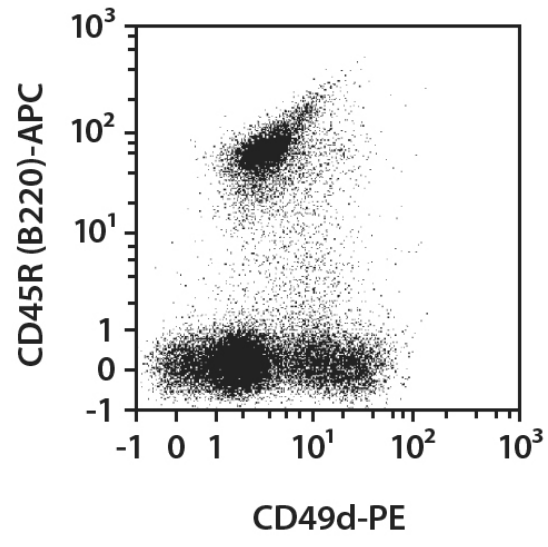
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

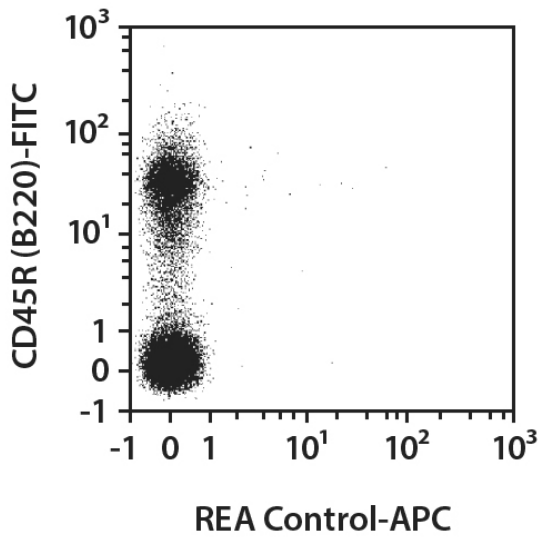
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

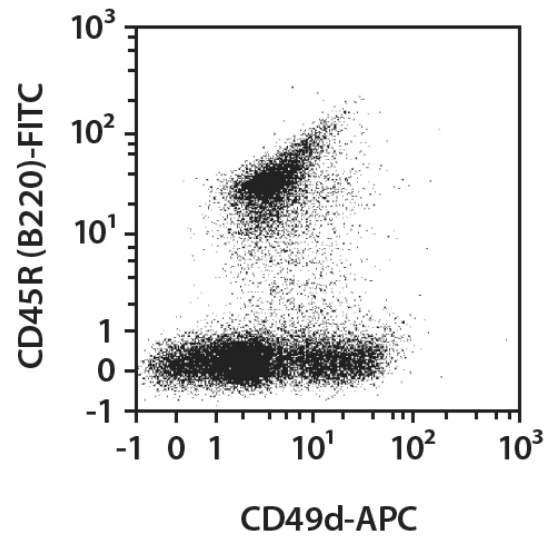
Examples of immunofluorescent staining

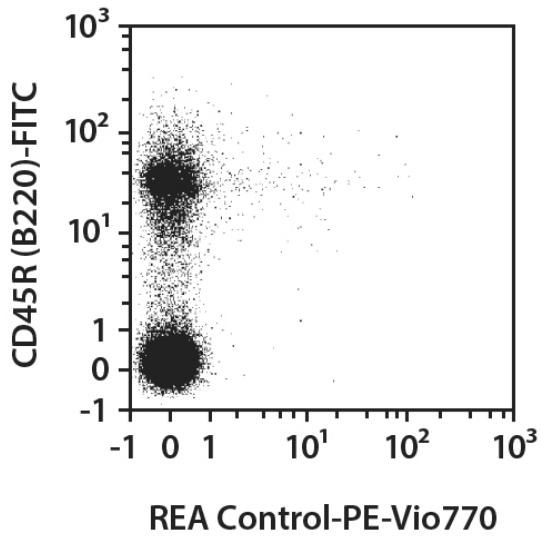
Splenocytes from BALB/c mice were stained with CD49d antibodies or with the corresponding REA Control antibodies (left image) as well as with CD45R (B220) antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

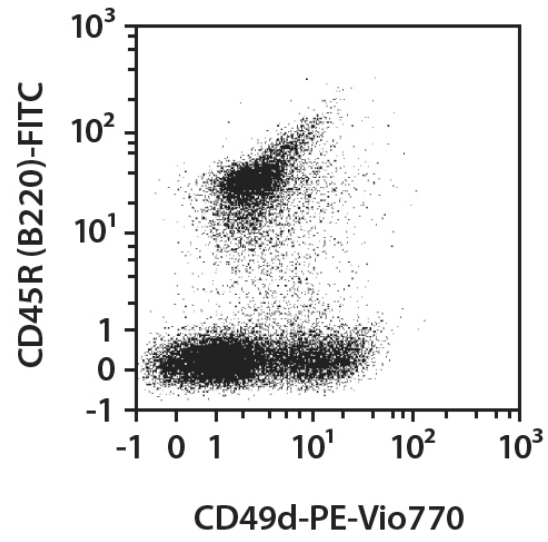


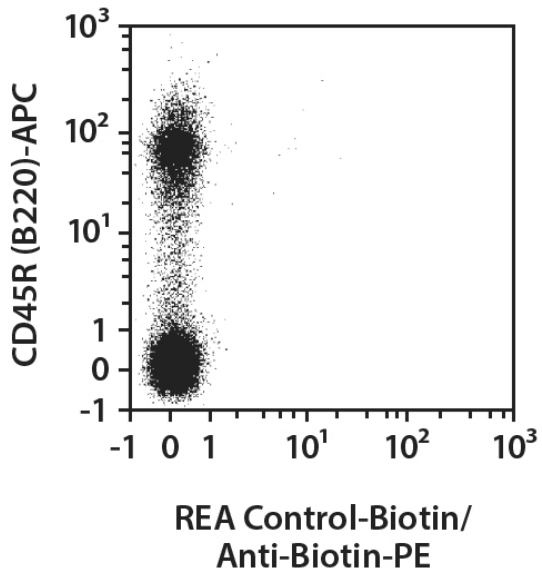


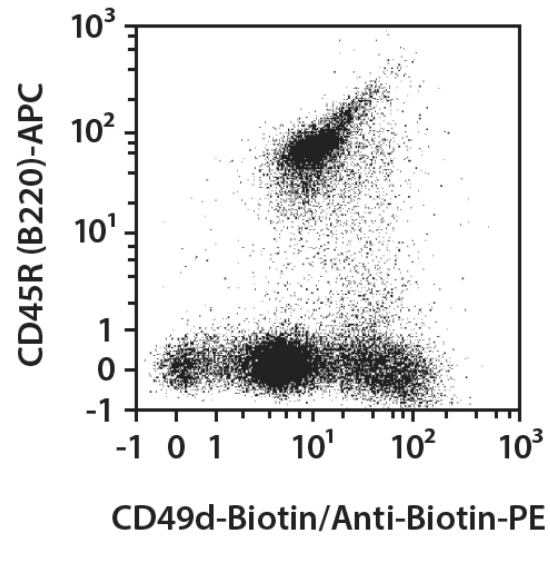












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