CD49d antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD49d-FITC	for 30 tests	130-099-720
CD49d-FITC	for 100 tests	130-093-283
CD49d-PE	for 30 tests	130-099-691
CD49d-PE	for 100 tests	130-093-282
CD49d-APC	for 30 tests	130-099-226
CD49d-APC	for 100 tests	130-093-281
CD49d-VioBlue	for 30 tests	130-099-680
CD49d-VioBlue	for 100 tests	130-099-681
CD49d-PE-Vio770	for 30 tests	130-104-326
CD49d-PE-Vio770	for 100 tests	130-104-277
CD49d-Biotin	for 30 tests	130-100-309
CD49d-Biotin	for 100 tests	130-093-280
CD49d pure	100 µg in 1 mL	130-093-279

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	MZ18-24A9
Isotype	mouse IgG2bκ
Isotype control	Mouse IgG2b – isotype control antibodies
Alternative names of antigen	ITGA4, IA4, VLA-4α
Molecular mass of antigen [kDa]	111
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)
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	Antibodies 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.

CD49d, also known as the α4 integrin chain or VLA-4α chain, is a 150 kDa cell adhesion protein that is directly involved in mononuclear leukocyte trafficking. Dimerized with β7 integrin, the resulting α4β7 integrin binds VCAM-1 (CD106), MAdCAM-1, and fibronectin to facilitate the rolling of leukocytes along vascular epithelium. In T cells, on ligation of MAdCAM-1, α4β7 integrin also provides costimulation of T cell receptor/CD3–mediated signaling.
CD49d is expressed on most mononuclear leukocytes, both in circulation or in lymphoid or other tissues, as well as on eosinophils and basophils.

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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (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.

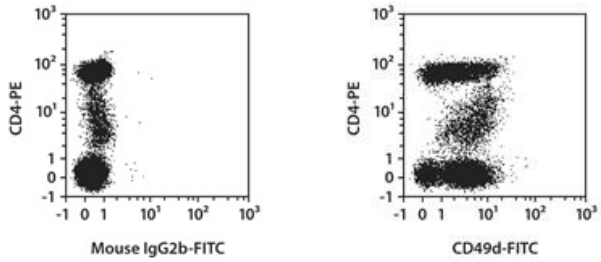
Protocol for cell surface staining

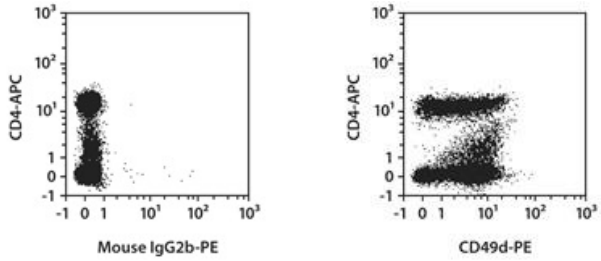
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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).

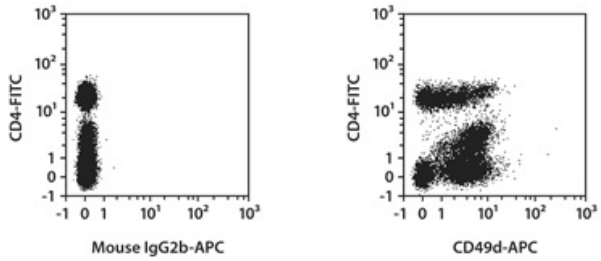
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

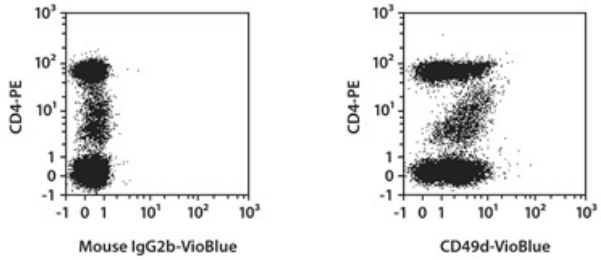
Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with CD49d antibodies or with the corresponding isotype control antibodies (left image) as well as with CD4 antibodies and analyzed by flow cytometry. 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 tandems.


CD4-PE
Mouse IgG2b-FITC
CD49d-FITC


CD4-APC
Mouse IgG2b-PE
CD49d-PE


CD4-FITC
Mouse IgG2b-APC
CD49d-APC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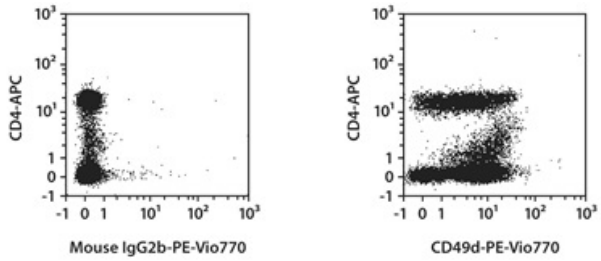
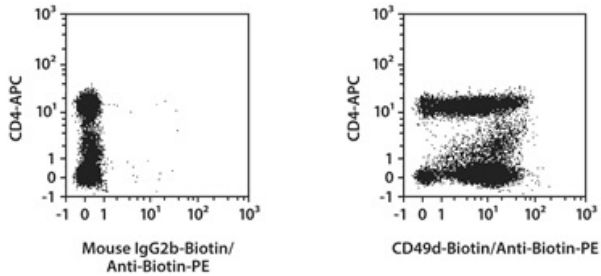
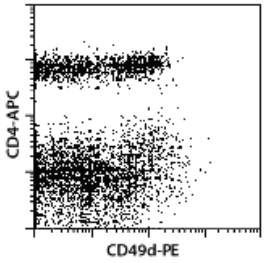
Mouse IgG2b-VioBlue
CD49d-VioBlue

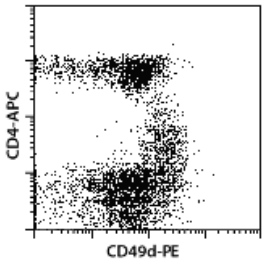

CD4-APC
Mouse IgG2b-PE-Vio770
CD49d-PE-Vio770


CD4-APC
Mouse IgG2b-Biotin/
Anti-Biotin-PE
CD49d-Biotin/Anti-Biotin-PE

Rhesus monkey PBMCs were stained with CD49d-PE and CD4-APC and analyzed by flow cytometry



Cynomolgus monkey PBMCs were stained with CD49d-PE and CD4-APC and analyzed by flow cytometry.



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 | **www.miltenyibiotec.com**
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