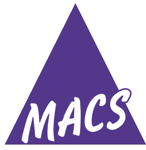

CD49d antibodies
human

CD49d-FITC	130-093-283
CD49d-PE	130-093-282
CD49d-APC	130-093-281
CD49d-Biotin	130-093-280
CD49d pure	130-093-279

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1. Description

Components 1 mL CD49d antibodies, human: monoclonal CD49d antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Clone MZ18-24A9 (isotype: mouse 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.

1.1 Background information

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[1]. Dimerized with β7 integrin, the resulting α4β7 integrin binds VCAM-1 (CD106), MAdCAM-1, and fibronectin[2] to facilitate the rolling of leukocytes along vascular epithelium. In T cells, on ligation of MAdCAM-1, α4β7 integrin also provides co-stimulation of T cell receptor/CD3–mediated signaling.[3]
CD49d is expressed on a broad range of cells, including T lymphocytes, B cells, monocytes, as well as eosinophils and basophils.[2] Leukocyte extravasation contributes to the pathogenesis of a number of chronic inflammation and autoimmune diseases, such as Crohn's disease and rheumatoid arthritis.

1.2 Applications

- Identification and enumeration of CD49d$^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human T cells can be isolated by using, for example, CD3 MicroBeads, human (# 130-050-101). Rhesus monkey (*Macaca mulatta*) cells can be isolated by using the CD3 MicroBead Kit, non-human primate (# 130-092-012).

1.3 Recommended antibody dilution

For antibody labeling of human and non-human primate cells.

CD49d conjugate	FITC	PE	APC	Biotin
Flow cytometry[a]				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10^7 cells/100 µL of buffer.

- Cross-reactivity: The CD49d antibody is tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*).

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. 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) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-CD49d-Biotin.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485).
- (Optional) CD4-FITC, human (# 130-080-501), CD4-PE, human (# 130-091-231), or CD4-APC, human (# 130-091-232).
- (Optional) Propidium iodide (PI) 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.

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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 CD49d antibody.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

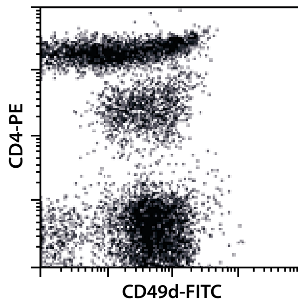
7. (Optional) If CD49d-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

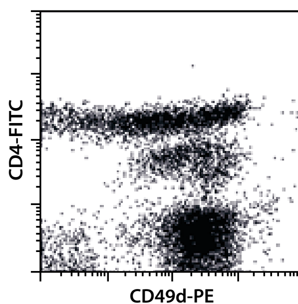
3. Examples of immunofluorescent staining with CD49d antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD49d antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Cells stained with CD49d-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856). All samples were also stained with either CD4-PE or CD4-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

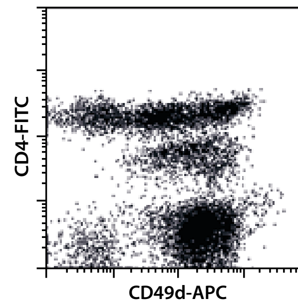
(a) Human PBMCs stained with CD49d-FITC and CD4-PE.



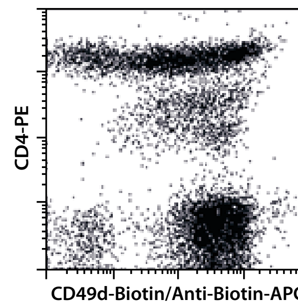
(b) Human PBMCs stained with CD49d-PE and CD4-FITC.



(c) Human PBMCs stained with CD49d-APC and CD4-FITC.

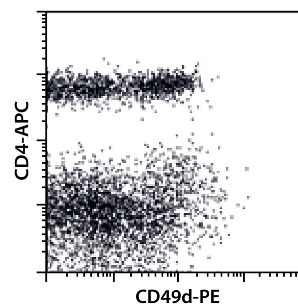


(d) Human PBMCs stained with CD49d-Biotin, Anti-Biotin-APC, and CD4-PE.



Rhesus monkey PBMCs were stained with CD49d-PE and CD4-APC (e) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(e) Rhesus monkey PBMCs stained with CD49d-PE.



4. References

1. Rose, D. M. (2006) The role of the α4 integrin-paxillin interaction in regulating leukocyte trafficking. Exp. Mol. Med. 38: 191–195.
2. Lobb, R. R. and Hemler, M. E. (1994) The pathophysiologic role of α4 integrins *in vivo*. J. Clin. Invest. 94: 1722–1728.
3. Lehnert, K. *et al.* (1998) MAdCAM-1 costimulates T cell proliferation exclusively through integrin alpha4beta7, whereas VCAM-1 and CS-1 peptide use alpha4beta1: evidence for "remote" costimulation and induction of hyperresponsiveness to B7 molecules. Eur. J. Immunol. 28: 3605–3615.

All protocols and data sheets are available at www.miltenyibiotec.com.

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