CD54 (ICAM-1) 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.
CD54 (ICAM-1)-PE	for 30 tests	130-103-909
CD54 (ICAM-1)-PE	for 100 tests	130-103-839
CD54 (ICAM-1)-APC	for 30 tests	130-103-910
CD54 (ICAM-1)-APC	for 100 tests	130-103-840
CD54 (ICAM-1)-PE-Vio615	for 30 tests	130-107-511
CD54 (ICAM-1)-PE-Vio615	for 100 tests	130-107-457
CD54 (ICAM-1)-PE-Vio770	for 30 tests	130-104-063
CD54 (ICAM-1)-PE-Vio770	for 100 tests	130-104-031
CD54 (ICAM-1)-Biotin	for 30 tests	130-103-911
CD54 (ICAM-1)-Biotin	for 100 tests	130-103-841

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	CD54 (ICAM-1)
Clone	REA266
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	ICAM-1, BB2, P3.58
Molecular mass of antigen [kDa]	55
Distribution of antigen	monocytes, macrophages, dendritic cells, endothelial 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 REA266 recognizes human CD54, a 85–110 kDa type I transmembrane glycoprotein, which also known as intercellular adhesion molecule 1 (ICAM-1). CD54 is continuously present in low concentrations in the membranes of monocytes/macrophages, lymphocytes, activated endothelial cells, granulocytes, and dendritic cells. Its expression is regulated by inflammatory cytokines and can

be induced by interleukin-1 and tumor necrosis factor. CD54 is a ligand for LFA-1, a receptor found on leukocytes. When activated, leukocytes bind to endothelial cells via CD54/LFA-1 and then transmigrate into tissues. CD54 has been shown to interact with CD11a, EZR, and CD18. More recently, CD54 has been characterized as a site for the cellular entry of human rhinovirus.
Additional information: Clone REA266 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.

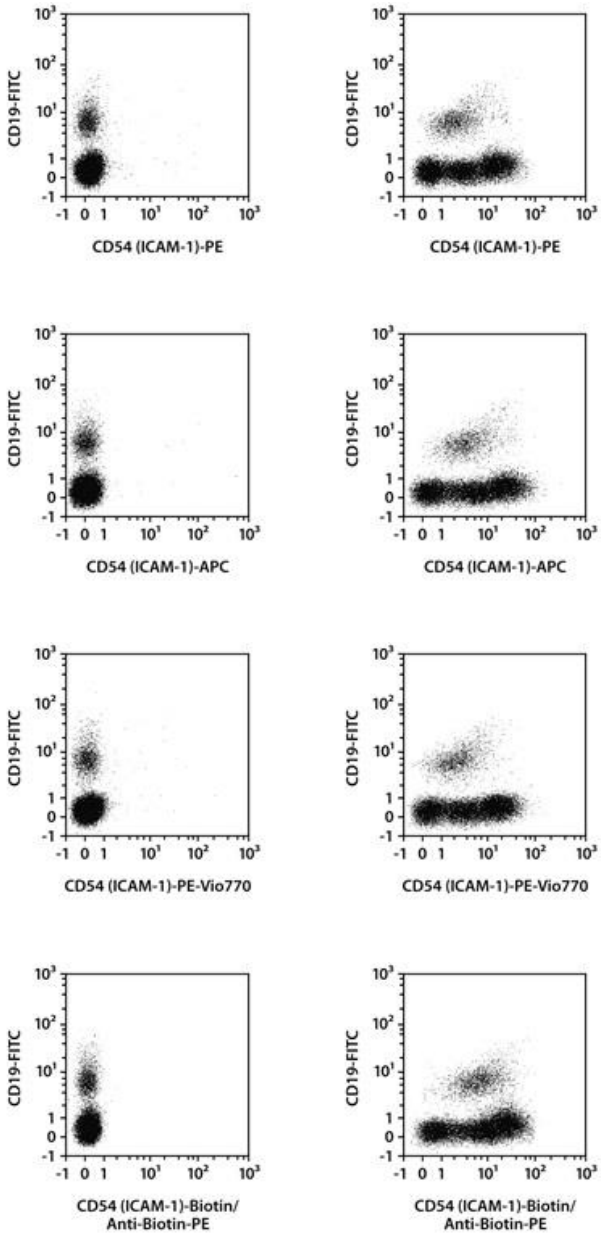
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 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 CD54 (ICAM-1) antibodies as well as with CD19 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. 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 tandem conjugates.



References

1. **Simmons, D. *et al.*** (1988) ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature 331(6157): 624–627.
2. **Greve, J. M. *et al.*** (1989) The major human rhinovirus receptor is ICAM-1. Cell 56(5): 839–847.
3. **Shimaoka, M. *et al.*** (2003) Structures of the α L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. Cell 112(1): 99–111.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are either trademarks or registered trademarks of Miltenyi Biotec GmbH. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.