CD154 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.
CD154-FITC	for 30 tests	130-109-548
CD154-FITC	for 100 tests	130-109-469
CD154-PE	for 30 tests	130-109-550
CD154-PE	for 100 tests	130-109-471
CD154-VioBlue	for 30 tests	130-109-549
CD154-VioBlue	for 100 tests	130-109-470
CD154-PE-Vio770	for 30 tests	130-109-552
CD154-PE-Vio770	for 100 tests	130-109-473
CD154-PerCP-Vio700	for 30 tests	130-109-553
CD154-PerCP-Vio700	for 100 tests	130-109-474
CD154-Biotin	for 30 tests	130-109-547
CD154-Biotin	for 100 tests	130-109-468

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	CD154
Clone	REA238
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	CD40L, CD40-L, gp39, T-BAM, TRAP, Ly-62, HIGM1, IGM
Molecular mass of antigen [kDa]	29
Cross-reactivity	cynomolgus monkey (*Macaca fascicularis*), rhesus monkey (*Macaca mulatta*)
Distribution of antigen	basophils, dendritic cells, lymphocytes, macrophages, mast cells, monocytes, NK cells, T 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 REA238 recognizes the human CD154 antigen, a 39 kDa transmembrane glycoprotein, also known as CD40L, gp39, T-BAM, TRAP, or Ly-62, which is a proinflammatory and prothrombotic ligand in the tumor necrosis factor family. CD154 is transiently up-regulated on activated $CD4^+$ T cells and plays an important role as a costimulatory molecule in T cell/antigen-presenting cell interactions through ligation of CD40. It mediates B cell proliferation in the absence of co-stimulus as well as IgE production in the presence of IL-4.
Additional information: Clone REA238 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.

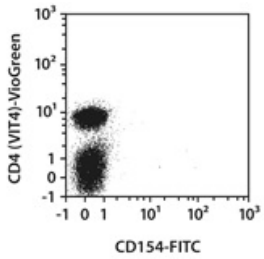
Protocol for cell surface staining

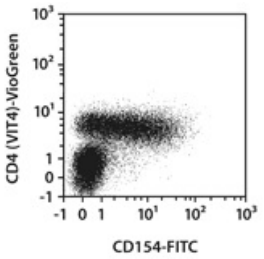
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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), either left unstimulated (left image) or stimulated with CytoStim and 1 µg/mL CD40 for 16 hours, were stained with CD154 antibodies as well as with CD4 (VIT4) antibodies. Cells were 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.


CD4 (VIT4)-VioGreen
CD154-FITC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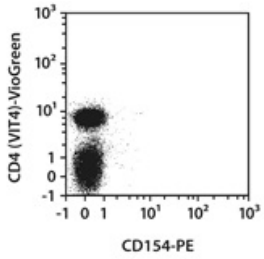
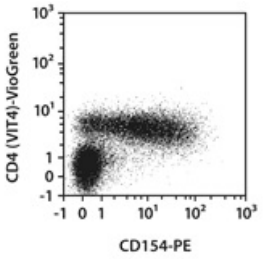
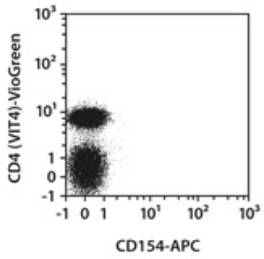
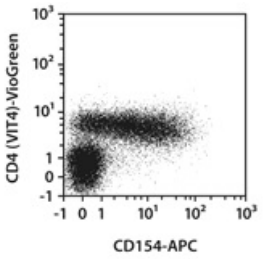
CD154-FITC


CD4 (VIT4)-VioGreen
CD154-PE


CD4 (VIT4)-VioGreen
CD154-PE


CD4 (VIT4)-VioGreen
CD154-APC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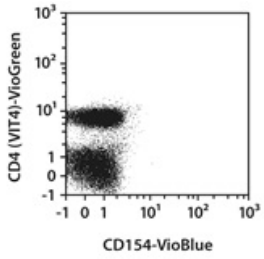
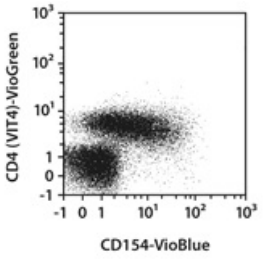
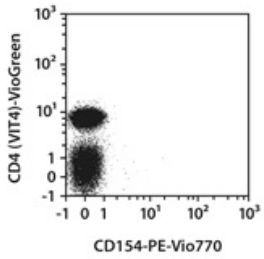
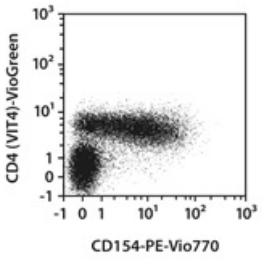
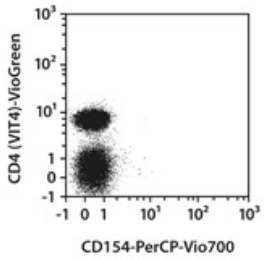
CD154-APC

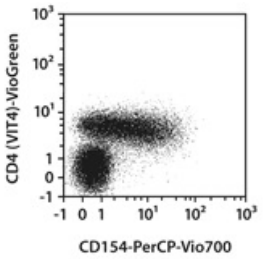

CD4 (VIT4)-VioGreen
CD154-VioBlue

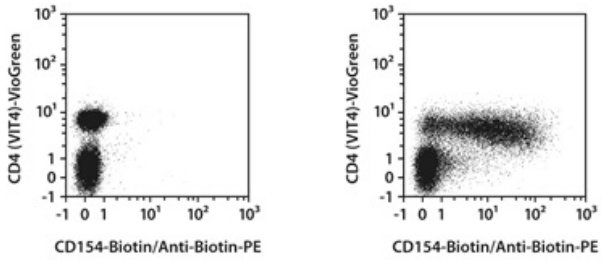

CD4 (VIT4)-VioGreen
CD154-VioBlue


CD4 (VIT4)-VioGreen
CD154-PE-Vio770


CD4 (VIT4)-VioGreen
CD154-PE-Vio770


CD4 (VIT4)-VioGreen
CD154-PerCP-Vio700


CD4 (VIT4)-VioGreen
CD154-PerCP-Vio700



References

1. **Hollenbaugh, D. *et al.*** (1992) The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J. 11(12): 4313–4321.
2. **Furman, M. *et al.*** (2004) Release of soluble CD40L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. J. Am. Coll. Cardiol. 43(12): 2319–2325.
3. **Frentsch, M. *et al.*** (2005) Direct access to $CD4^+$ T cells specific for defined antigens according to CD154 expression. Nat. Med. 11: 1118–1124.

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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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