CD154 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.
CD154-PE	30 µg in 200 µL	130-111-360
CD154-PE	150 µg in 1 mL	130-111-172
CD154-APC	30 µg in 200 µL	130-111-361
CD154-APC	150 µg in 1 mL	130-111-173
CD154-VioBright 515	30 µg in 200 µL	130-111-362
CD154-VioBright 515	150 µg in 1 mL	130-111-174
CD154-Biotin	30 µg in 200 µL	130-111-359
CD154-Biotin	150 µg in 1 mL	130-111-171

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	REA785
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	CD40LG, CD40L, HIGM1, IGM, IMD3, Ly-62, T-BAM, TRAP, TNFSF5, gp39
Entrez Gene ID	21947
Molecular mass of antigen [kDa]	29
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 REA785 specifically recognizes the mouse CD154 antigen, a 39 kDa transmembrane glycoprotein also known as CD40L, gp39, T-BAM, TRAP, or Ly-62. 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. Due to its transient expression within hours after activation, CD154 can be used as a marker for activated antigen-specific $CD4^+$ T cells. Adding a CD40-blocking antibody during the stimulation of cell suspensions prevents the down-regulation of CD154. Blocking of CD40 is not required for intracellular detection of CD154 expression or if a pure population of enriched T cells is used.

Additional information: Clone REA785 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.

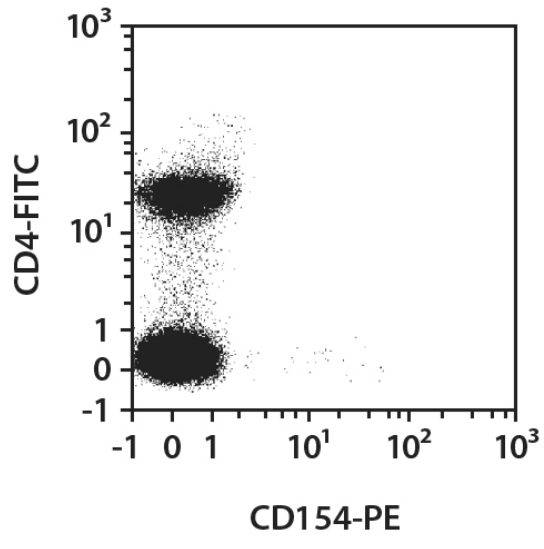
Protocol for cell surface staining

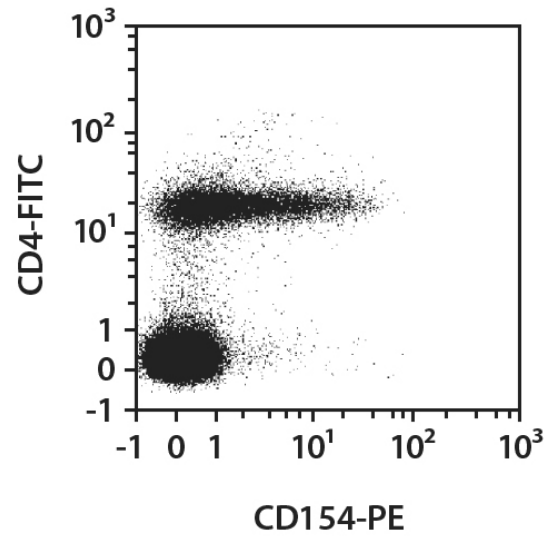
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

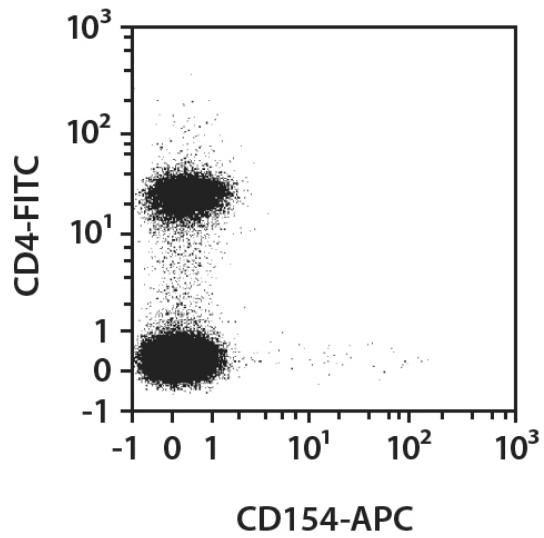
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

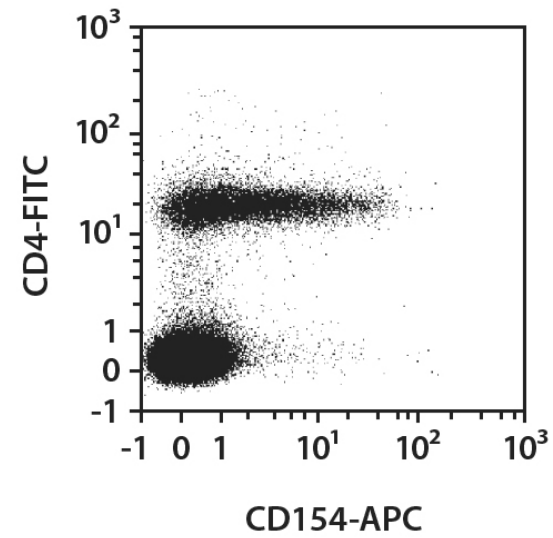
Examples of immunofluorescent staining

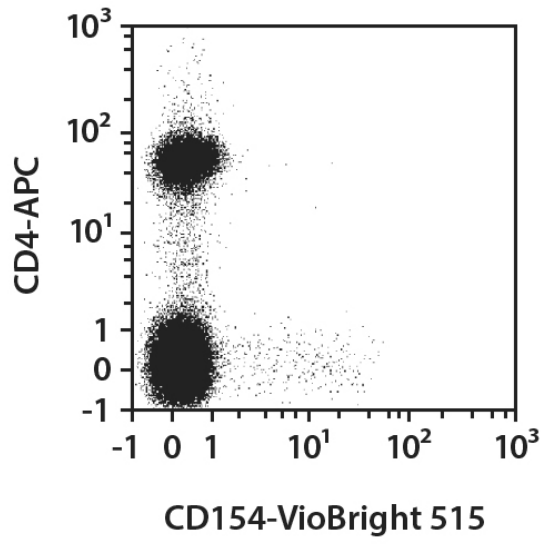
Splenocytes from BALB/c mice were either left unstimulated (left image) or stimulated with CD3ε/CD28/CD40 antibodies for 6 hours. Cells were then stained with CD154 antibodies as well as with CD4 antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

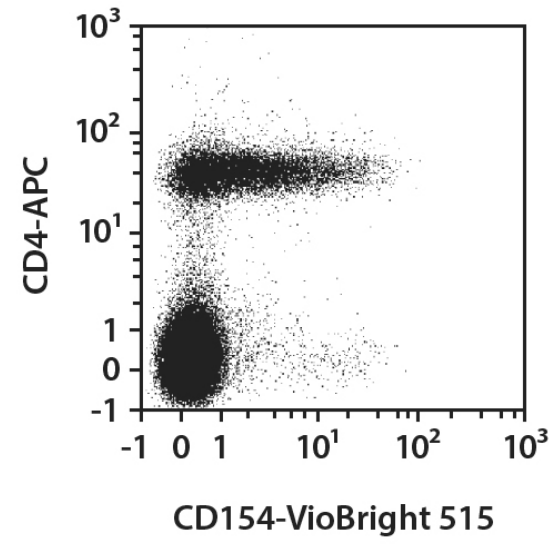


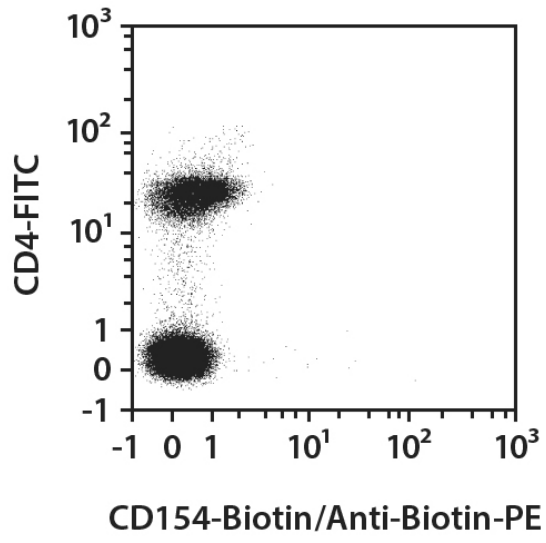


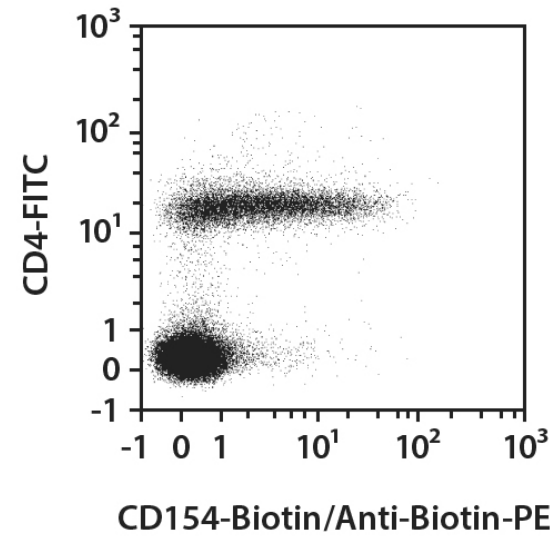












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Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.