CD4 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.
CD4-VioBright FITC	for 30 tests	130-109-536
CD4-VioBright FITC	for 100 tests	130-109-457
CD4-APC	for 30 tests	130-109-531
CD4-APC	for 100 tests	130-109-452
CD4-VioGreen	for 30 tests	130-109-535
CD4-VioGreen	for 100 tests	130-109-456
CD4-PE-Vio615	for 30 tests	130-109-537
CD4-PE-Vio615	for 100 tests	130-109-458
CD4-PerCP-Vio700	for 30 tests	130-109-534
CD4-PerCP-Vio700	for 100 tests	130-109-455
CD4-Biotin	for 30 tests	130-109-529
CD4-Biotin	for 100 tests	130-109-450

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	CD4
Clone	REA623
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	T4, Leu-3, CD4mut
Molecular mass of antigen [kDa]	48
Distribution of antigen	monocytes, T cells, thymocytes, T helper 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 REA623 recognizes the human CD4 antigen, a 55 kDa single-pass type I membrane protein, also known as T4/Leu-3. CD4 is highly expressed on T helper cells and at a lower level on monocytes

and dendritic cells. It is involved in the recognition of MHC class II/peptide complexes by the TCR heterodimers and is the receptor for the human immunodeficiency virus (HIV).
Additional information: Clone REA623 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.

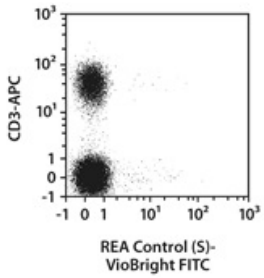
Protocol for cell surface staining

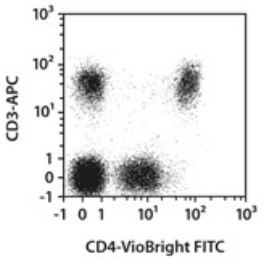
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher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

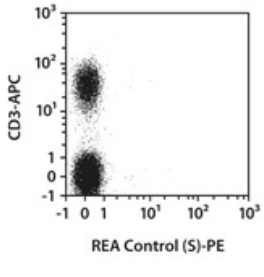
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

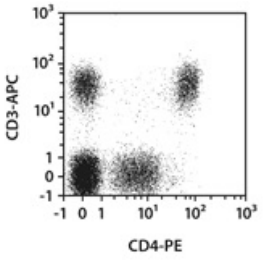
Examples of immunofluorescent staining

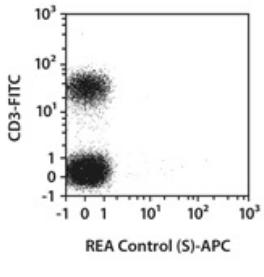
Human peripheral blood mononuclear cells (PBMCs) were stained with CD4 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding 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.

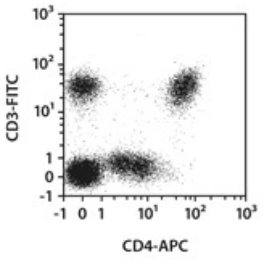

CD3-APC
REA Control (S)-
VioBright FITC


CD3-APC
CD4-VioBright FITC


CD3-APC
REA Control (S)-PE


CD3-APC
CD4-PE


CD3-FITC
REA Control (S)-APC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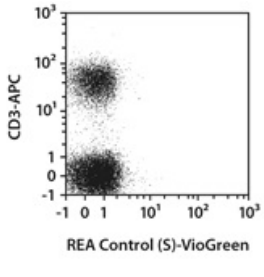
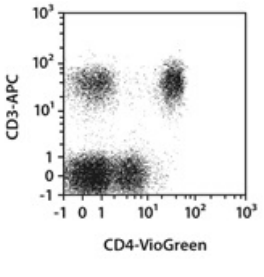
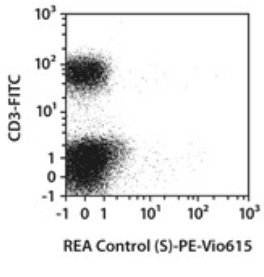
CD4-APC


CD3-APC
REA Control (S)-VioGreen


CD3-APC
CD4-VioGreen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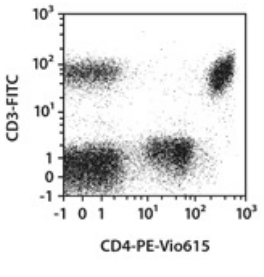
REA Control (S)-PE-Vio615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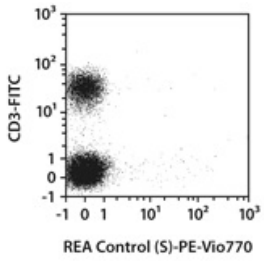
CD4-PE-Vio615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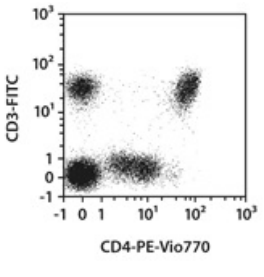
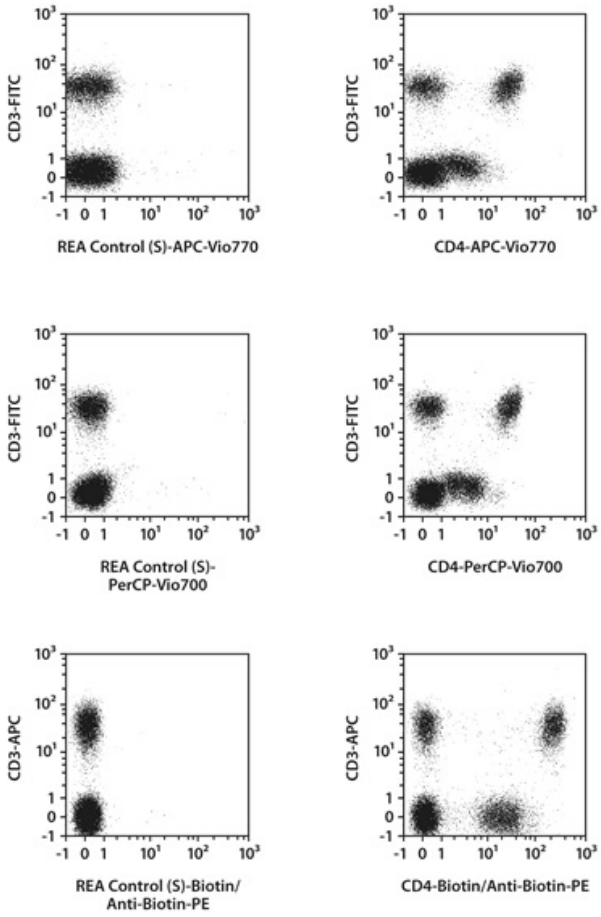

CD3-FITC
CD4-PE-Vio770



References

1. **Maddon, P. J. *et al.*** (1985) The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. Cell 42(1): 93–104.
2. **Barclay, A. N. *et al.*** (1993) CD4 and the immunoglobulin superfamily. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 342(1299): 7–12.
3. **Tebas, P. *et al.*** (2014) Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N. Engl. J. Med. 370(10): 901–910.

Warranty

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