CD4 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD4-VioBright FITC	9 µg in 300 µL	130-109-498
CD4-VioBright FITC	30 µg in 1 mL	130-109-419
CD4-PE	9 µg in 300 µL	130-109-493
CD4-PE	30 µg in 1 mL	130-109-414
CD4-APC	9 µg in 300 µL	130-109-494
CD4-APC	30 µg in 1 mL	130-109-415
CD4-VioBlue	9 µg in 300 µL	130-110-411
CD4-VioBlue	30 µg in 1 mL	130-110-310
CD4-VioGreen	9 µg in 300 µL	130-109-492
CD4-VioGreen	30 µg in 1 mL	130-109-413
CD4-PE-Vio615	30 µg in 1 mL	130-109-420
CD4-PE-Vio770	9 µg in 300 µL	130-109-495
CD4-PE-Vio770	30 µg in 1 mL	130-109-416
CD4-APC-Vio770	9 µg in 300 µL	130-109-496
CD4-APC-Vio770	30 µg in 1 mL	130-109-417
CD4-PerCP-Vio700	9 µg in 300 µL	130-109-497
CD4-PerCP-Vio700	30 µg in 1 mL	130-109-418
CD4-Biotin	9 µg in 300 µL	130-109-491
CD4-Biotin	30 µg in 1 mL	130-109-412

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	REA604
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	L3T4, T4, Leu-3, Ly-4
Molecular mass of antigen [kDa]	48
Distribution of antigen	T cells, NKT cells, dendritic cells, thymocytes
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 REA604 recognizes the mouse CD4 antigen, a cell surface glycoprotein, which is a member of the immunoglobulin superfamily. It is also known as L3T4 or Leu-3. In mice, CD4 is expressed on T helper cells, regulatory T cells, and at lower levels on subpopulations of NKT cells and dendritic cells. It is furthermore detected on most thymocytes ($CD4^+CD8^+$ and $CD4^+CD8^-$ thymocytes). CD4 interacts with MHC class II molecules on the surface of antigen-presenting cells. It increases the avidity of the interaction between T cells and antigen-presenting or target cells.
Additional information: Clone REA604 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.

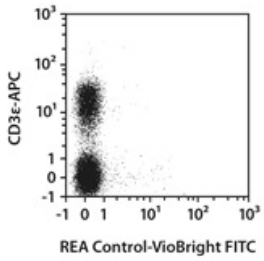
Protocol for cell surface staining

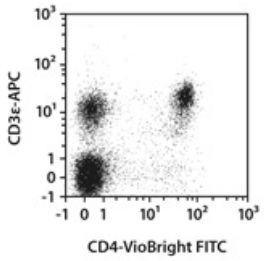
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

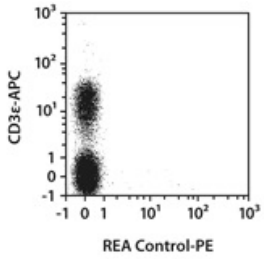
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

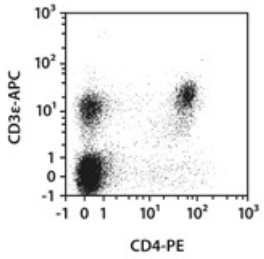
Examples of immunofluorescent staining

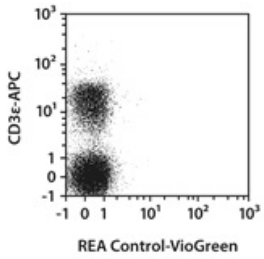
Splenocytes of BALB/c mice were stained with CD4 antibodies or with the corresponding REA Control antibodies (left images) as well as with CD3ε antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide.

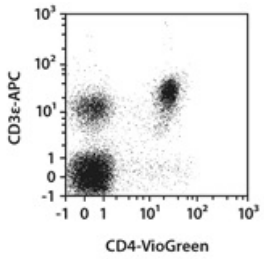

CD3ε-APC
REA Control-VioBright FITC

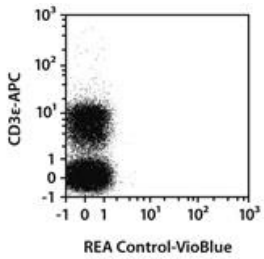

CD3ε-APC
CD4-VioBright FITC

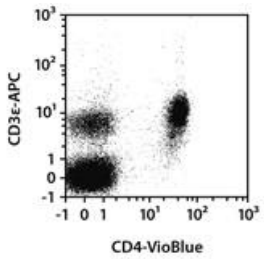

CD3ε-APC
REA Control-PE

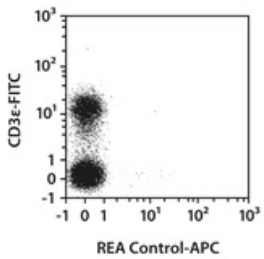

CD3ε-APC
CD4-PE

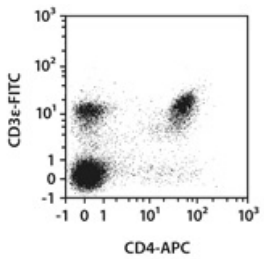

CD3ε-APC
REA Control-VioGreen

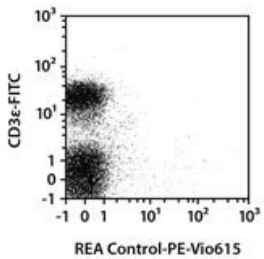

CD3ε-APC
CD4-VioGreen

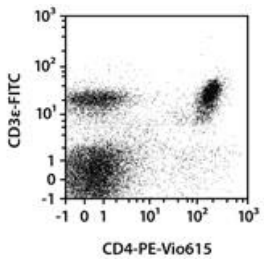

CD3ε-APC
REA Control-VioBlue

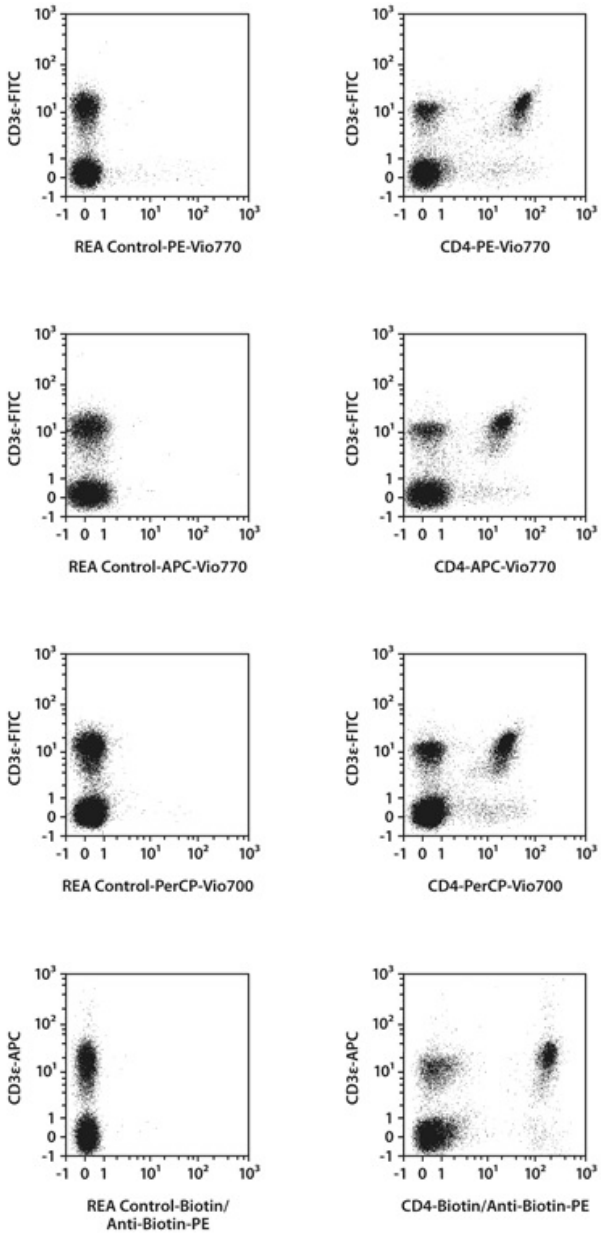

CD3ε-APC
CD4-VioBlue


CD3ε-FITC
REA Control-APC


CD3ε-FITC
CD4-APC


CD3ε-FITC
REA Control-PE-Vio615


CD3ε-FITC
CD4-PE-Vio615



References

1. **Tourvieille, B. *et al.*** (1986) Isolation and sequence of L3T4 complementary DNA clones: expression in T cells and brain. Science 234(4776): 610–614.
2. **Parnes, J. R. *et al.*** (1987) L3T4 and the immunoglobulin gene superfamily: new relationships between the immune system and the nervous system. Immunol. Rev. 100: 109–127.
3. **Bierer, B. E. *et al.*** (1989) The biologic roles of CD2, CD4, and CD8 in T-cell activation. Annu. Rev. Immunol. 7: 579–599.

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