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-FITC	9 µg in 300 µL	130-102-779
CD4-FITC	30 µg in 1 mL	130-102-541
CD4-PE	9 µg in 300 µL	130-102-780
CD4-PE	30 µg in 1 mL	130-102-619
CD4-APC	9 µg in 300 µL	130-102-782
CD4-APC	30 µg in 1 mL	130-102-597
CD4-VioBlue[1]	9 µg in 300 µL	130-102-774
CD4-VioBlue[1]	30 µg in 1 mL	130-102-456
CD4-VioGreen[1]	9 µg in 300 µL	130-102-777
CD4-VioGreen[1]	30 µg in 1 mL	130-102-444
CD4-PE-Vio770	9 µg in 300 µL	130-102-784
CD4-PE-Vio770	30 µg in 1 mL	130-102-411
CD4-APC-Vio770	9 µg in 300 µL	130-102-786
CD4-APC-Vio770	30 µg in 1 mL	130-102-179
CD4-PerCP-Vio700	30 µg in 1 mL	130-102-271
CD4-Biotin	9 µg in 300 µL	130-102-024
CD4-Biotin	30 µg in 1 mL	130-101-962

[1]Not recommended for cells that are labeled with MACS MicroBeads using the same antigen.

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	GK1.5
Isotype	rat IgG2bκ
Isotype control	Rat IgG2b – isotype control antibodies
Alternative names of antigen	L3T4, Ly-4, Leu-3, T4
Molecular mass of antigen [kDa]	48
Distribution of antigen	dendritic cells, granulocytes, Langerhans cells, lymphocytes, macrophages, monocytes, neutrophils, T cells, T helper cells, thymocytes

Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	With the exception of the VioGreen conjugate the antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone GK1.5 recognizes the mouse CD4 (L3T4) antigen. 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).

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) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (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.

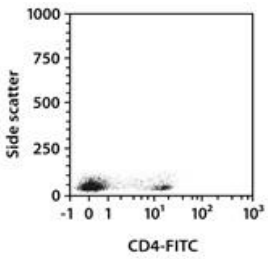
Protocol for cell surface staining

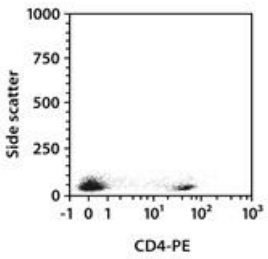
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher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

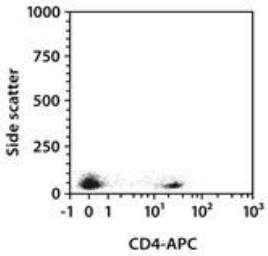
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

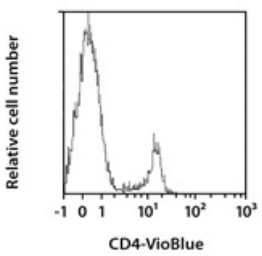
Examples of immunofluorescent staining

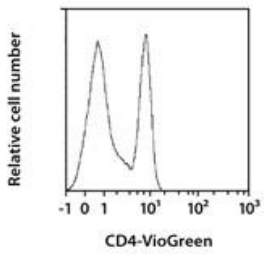
Mouse splenocytes were stained with CD4 antibodies and analyzed by flow cytometry. Cells stained with CD4-Biotin were stained with Anti-Biotin-PE in addition. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of CD4-PE-Vio770, CD4-APC-Vio770, CD4-PerCP-Vio700.

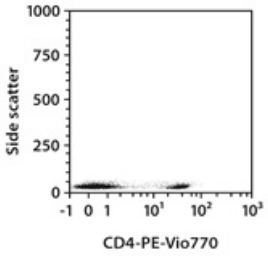


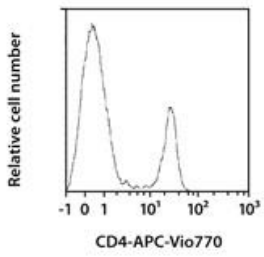


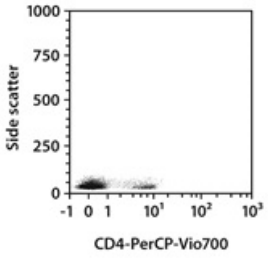


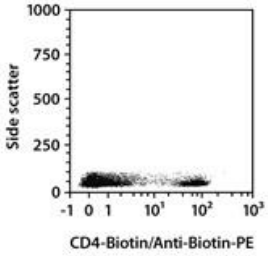












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