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-FITC	for 30 tests	130-098-206
CD4-FITC	for 100 tests	130-080-501
CD4-PE	for 30 tests	130-098-134
CD4-PE	for 100 tests	130-091-231
CD4-APC	for 30 tests	130-098-133
CD4-APC	for 100 tests	130-091-232
CD4-VioBlue	for 30 tests	130-099-683
CD4-VioBlue	for 100 tests	130-097-333
CD4-VioGreen	for 30 tests	130-106-712
CD4-VioGreen	for 100 tests	130-106-655
CD4-PerCP	for 30 tests	130-101-129
CD4-PerCP	for 100 tests	130-101-147
CD4-PE-Vio770	for 30 tests	130-100-452
CD4-PE-Vio770	for 100 tests	130-100-454
CD4-APC-Vio770	for 30 tests	130-100-455
CD4-APC-Vio770	for 100 tests	130-100-457
CD4-PerCP-Vio700	for 30 tests	130-103-863
CD4-PerCP-Vio700	for 100 tests	130-103-793
CD4-Biotin	for 100 tests	130-098-543

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	M-T466
Isotype	mouse IgG1
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	CD4mut, L3T4, Leu-3, T4
Molecular mass of antigen [kDa]	48
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)

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 FITC conjugate the antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

CD4 is a type I transmembrane glycoprotein involved in the recognition of MHC class II/peptide complexes by the TCR heterodimers. CD4 is highly expressed on T helper cells and at a lower level on monocytes and dendritic cells. The CD4 molecule is the receptor for the human immunodeficiency virus. The CD4 antibody recognizes the human CD4 antigen which is highly expressed on human T helper cells and thymocytes, and at lower levels on monocytes and dendritic cells. It is responsible for the recognition of the MHC class II antigen.

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, human (# 130-059-901) 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.

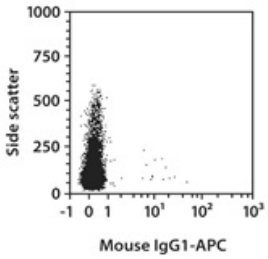
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 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(M-T466) antibodies or with the corresponding isotype control antibodies (left image) and 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.


1000
750
500
250
0
Side scatter
-1 0 1 10¹ 10² 10³
Mouse IgG1-APC








PBMCs from Rhesus monkey (A) and Cynomolgus monkey (B) were stained with CD4-FITC and analyzed by flow cytometry.

A:

B:

CHO cells were transfected with pMACS 4-IRES.II. A sample of the transfected cells was fixed and stained with CD4-FITC to evaluate transfection efficiency (transfected cells shown in green, cell nuclei in blue).

References

1. **Lamprecht, B. *et al.*** (2008) Aberrant expression of the Th2 cytokine IL-21 in Hodgkin lymphoma cells regulates STAT3 signaling and attracts Treg cells via regulation of MIP-3alpha. Blood 112(8): 3339–3347.
2. **Sacha, J. B. *et al.*** (2010) Synchronous Nat. Protoc. 5(2): 239–246.

Warranty

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