CD3 antibodies, human

For research use only

One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
CD3-APC	for 100 tests	130-113-125
CD3-FITC	for 30 tests	130-113-690
CD3-FITC	for 100 tests	130-113-128
CD3-PE	for 30 tests	130-113-691
CD3-PE	for 100 tests	130-113-129
CD3-APC	for 30 tests	130-113-687
CD3-VioBlue	for 30 tests	130-113-695
CD3-VioBlue	for 100 tests	130-113-133
CD3-VioBlue	for 500 tests	130-114-574
CD3-VioGreen	for 30 tests	130-113-696
CD3-VioGreen	for 100 tests	130-113-134
CD3-PerCP	for 30 tests	130-113-693
CD3-PerCP	for 100 tests	130-113-131
CD3-PE-Vio770	for 30 tests	130-113-692
CD3-PE-Vio770	for 100 tests	130-113-130
CD3-APC-Vio770	for 30 tests	130-113-688
CD3-APC-Vio770	for 100 tests	130-113-126
CD3-PerCP-Vio700	for 30 tests	130-113-694
CD3-PerCP-Vio700	for 100 tests	130-113-132
CD3-Biotin	for 30 tests	130-113-689
CD3-Biotin	for 100 tests	130-113-127

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	CD3
Clone	BW264/56
Isotype	mouse IgG2aκ
Isotype control	Mouse IgG2a – isotype control antibodies

Alternative names of antigen	CD3e, IMD18, T3E, TCRE
Entrez Gene ID	915, 916, 917
Molecular mass of antigen [kDa]	56
Distribution of antigen	NK cells, T 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.

The CD3 antibody recognizes the human CD3 antigen which is present on mature human T cells, thymocytes, and a subset of NK cells. CD3 is associated with the T cell receptor (TCR) and is responsible for its signal transduction. The CD3 antigen is a complex of five invariable chains: γ, δ, ε, ζ, and η. The epitope recognized by the antibody is located on the ε-chain of the CD3 complex.

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.

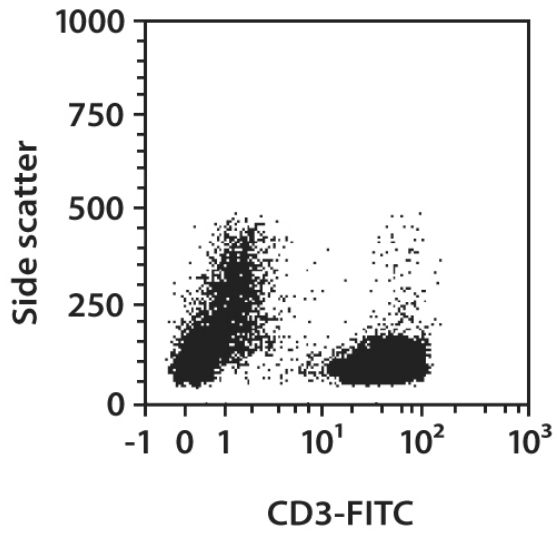
Protocol for cell surface staining

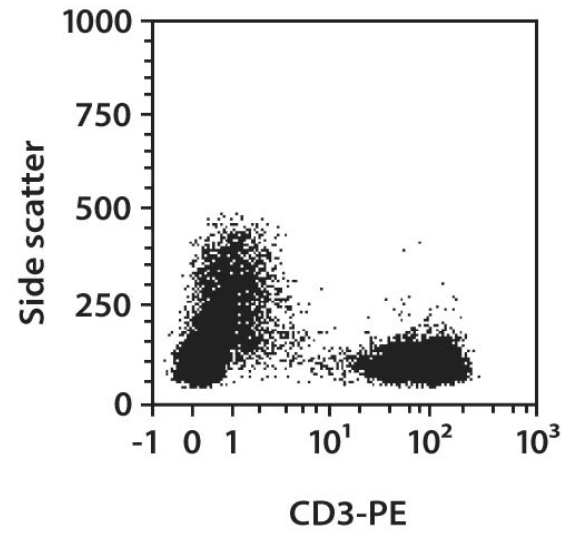
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

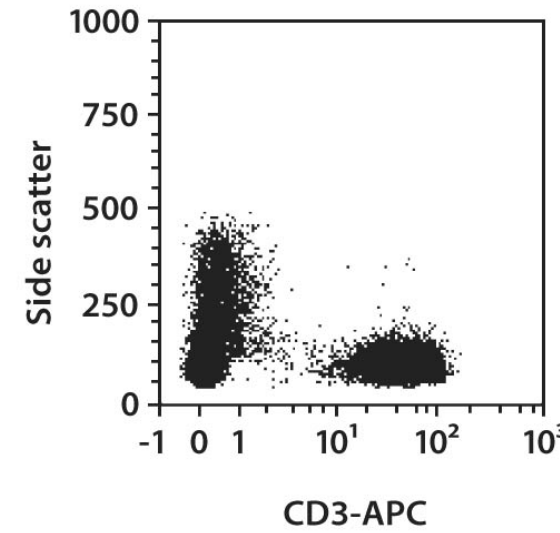
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

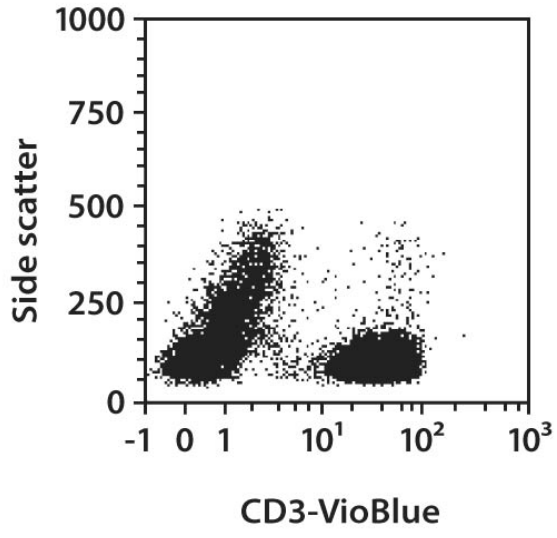
Examples of immunofluorescent staining

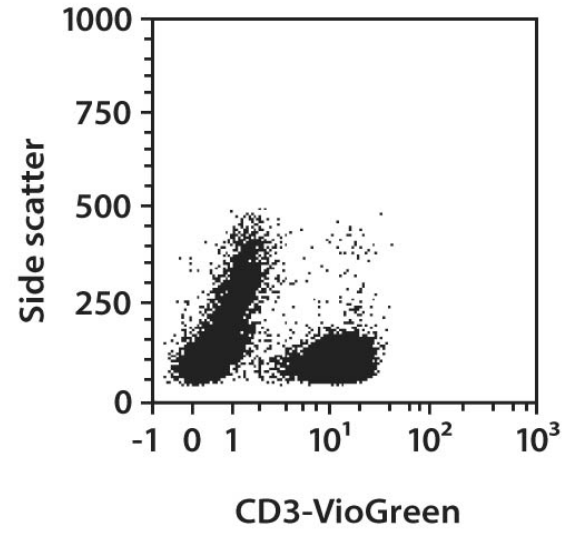
Human peripheral blood mononuclear cells (PBMCs) were stained with CD3 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

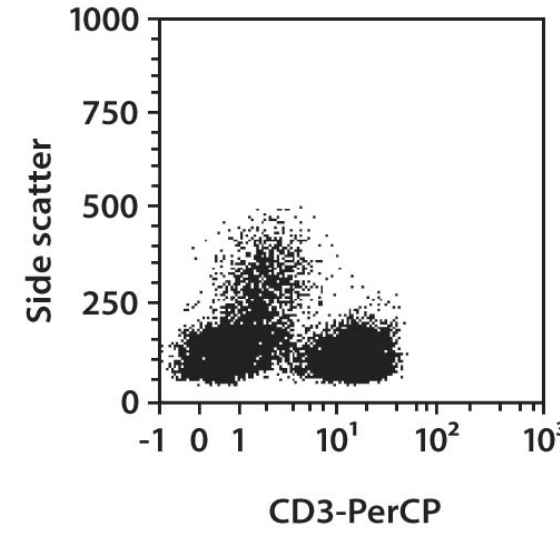


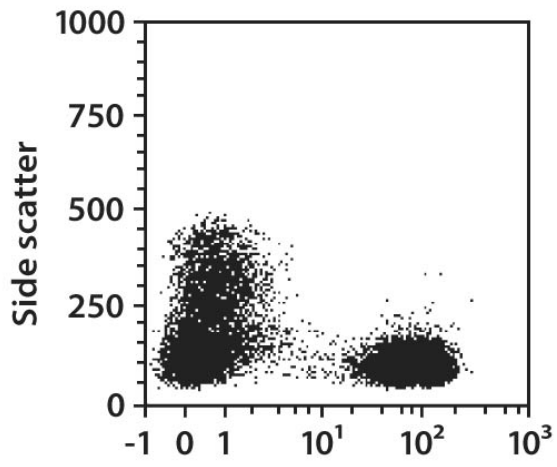


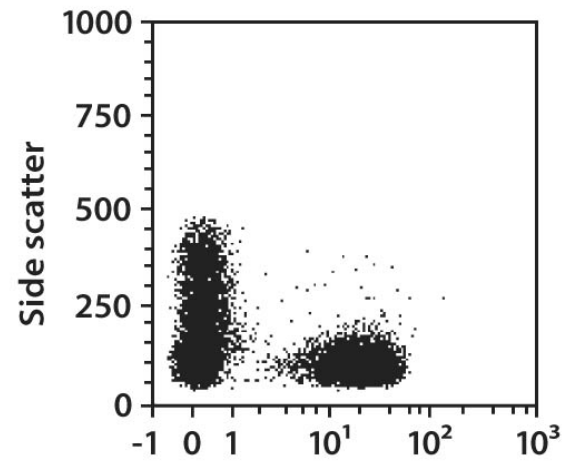


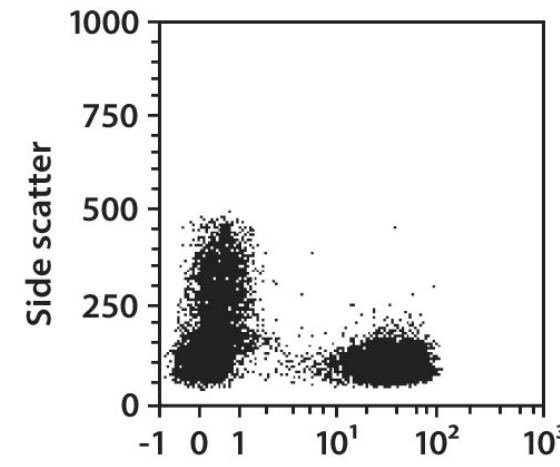


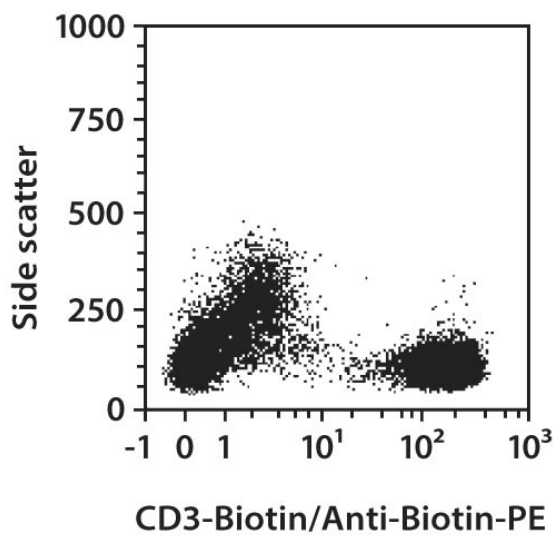












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