CD44 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.
CD44-FITC	for 30 tests	130-110-393
CD44-FITC	for 100 tests	130-110-292
CD44-APC	for 30 tests	130-110-395
CD44-APC	for 100 tests	130-110-294
CD44-VioBlue	for 30 tests	130-110-399
CD44-VioBlue	for 100 tests	130-110-298
CD44-PE-Vio770	for 30 tests	130-110-396
CD44-PE-Vio770	for 100 tests	130-110-295
CD44-APC-Vio770	for 30 tests	130-110-397
CD44-APC-Vio770	for 100 tests	130-110-296
CD44-PerCP-Vio700	for 30 tests	130-110-398
CD44-PerCP-Vio700	for 100 tests	130-110-297
CD44-Biotin	for 30 tests	130-110-392
CD44-Biotin	for 100 tests	130-110-291

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	CD44
Clone	REA690
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	CD44s, EMCR III, H-CAM, Pgp-1
Entrez Gene ID	960
Molecular mass of antigen [kDa]	79
Cross-reactivity	rhesus monkey (*Macaca mulatta*)
Distribution of antigen	bone marrow, cancer stem cells, CNS cells, endothelial cells, epithelial cells, kidney, leukocytes, lymphocytes, mesenchymal stem cells, myeloid cells, plasma cells, ES and iPS cells, red blood cells, skeletal muscle, skin, T cells

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

Clone REA690 recognizes the CD44 antigen. CD44 is a marker for many types of cancer stem cells (CSCs), including breast CSCs that possess higher tumorigenicity and metastatic potential, colorectal, pancreatic, and prostate CSCs. In addition, expression was observed in several cancers as well as on carcinoma cell lines. Here, CD44 plays a role in cancer cell migration and matrix adhesion in response to a cellular microenvironment, thus enhancing cellular aggregation and tumor cell growth. CD44 is also expressed on mesodermal cells, such as hematopoietic, fibroblastic, and glial cells. Additional information: Clone REA690 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.

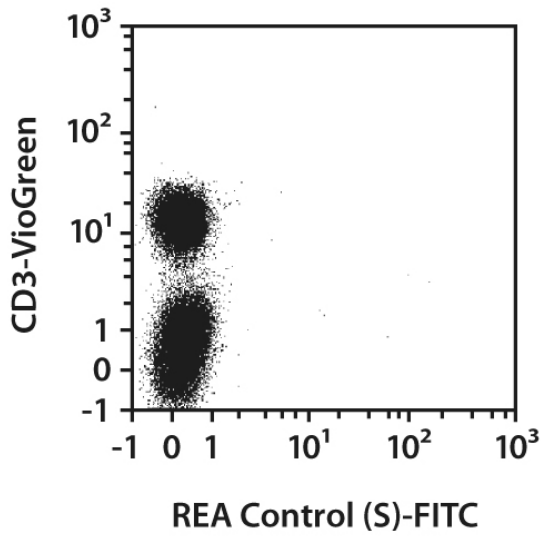
Protocol for cell surface staining

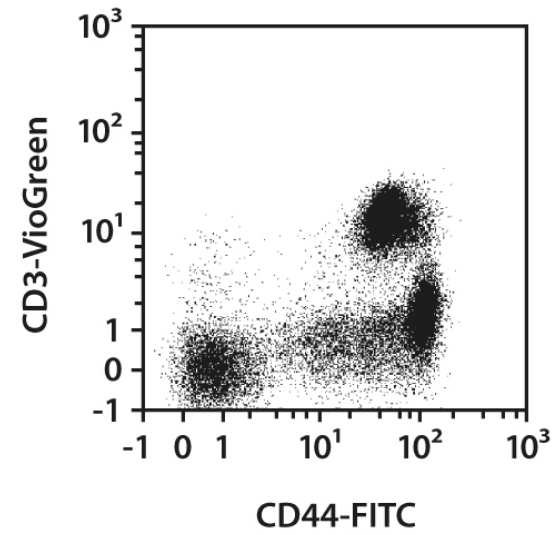
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher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

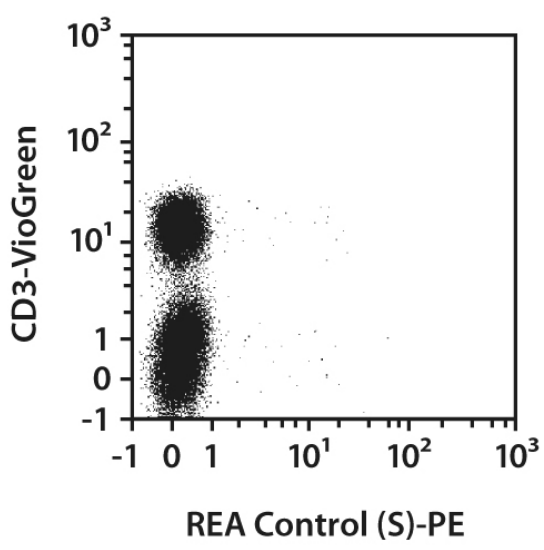
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

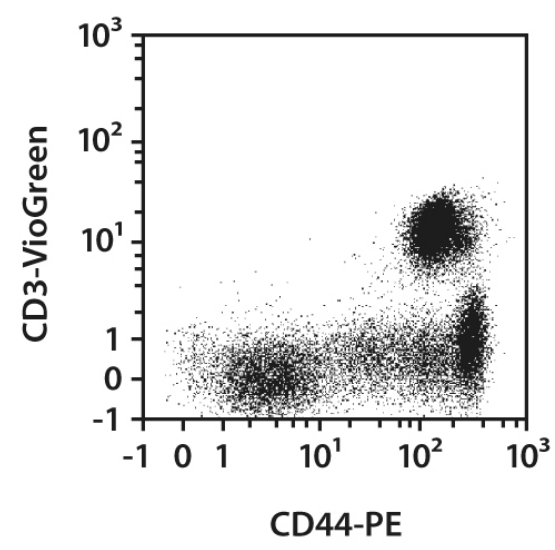
Examples of immunofluorescent staining

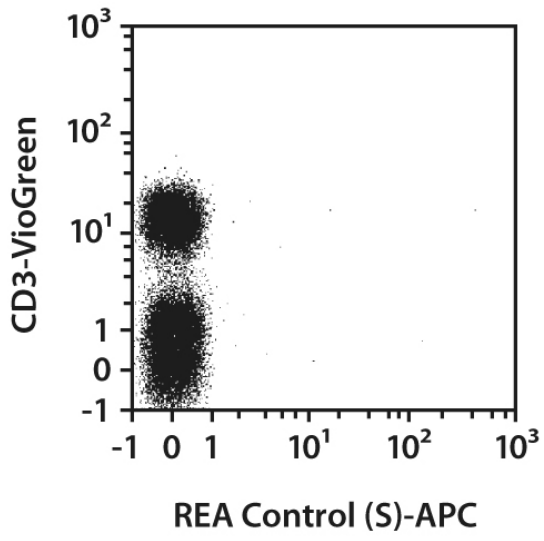
Human peripheral blood mononuclear cells (PBMCs) were stained with CD44 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding 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.

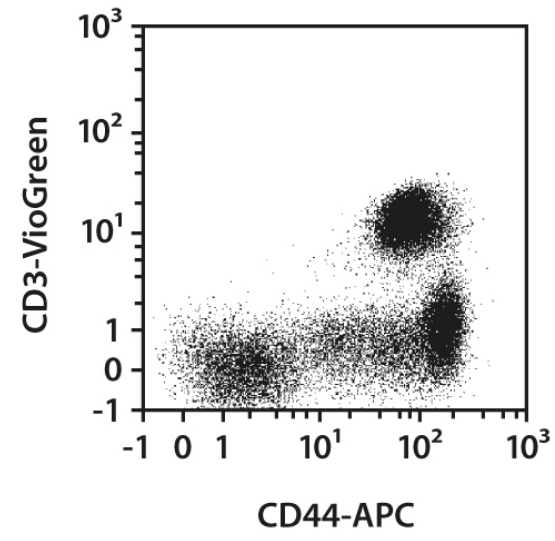

CD3-VioGreen
REA Control (S)-FITC

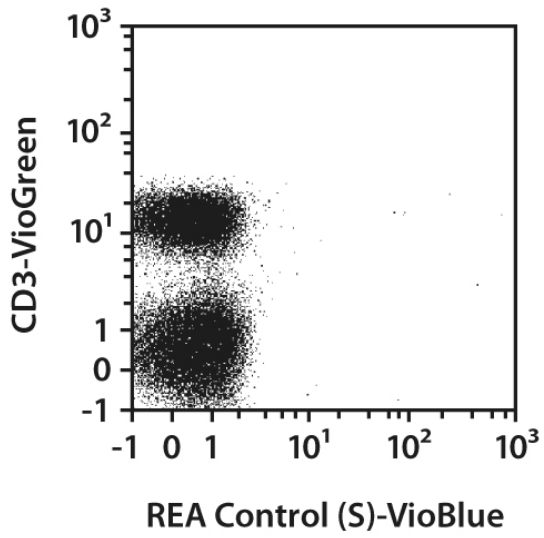

CD3-VioGreen
CD44-FITC

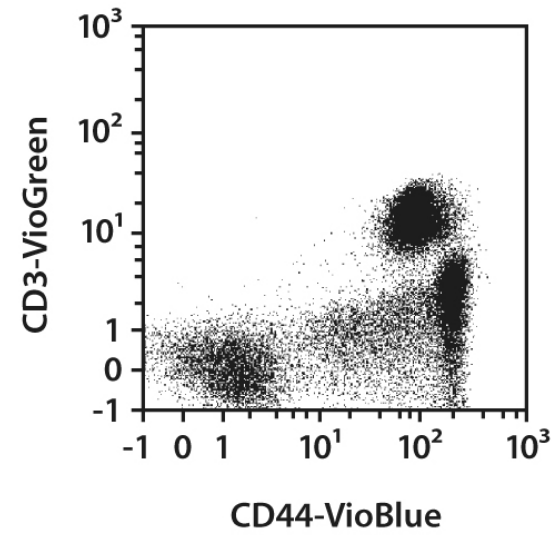

CD3-VioGreen
REA Control (S)-PE

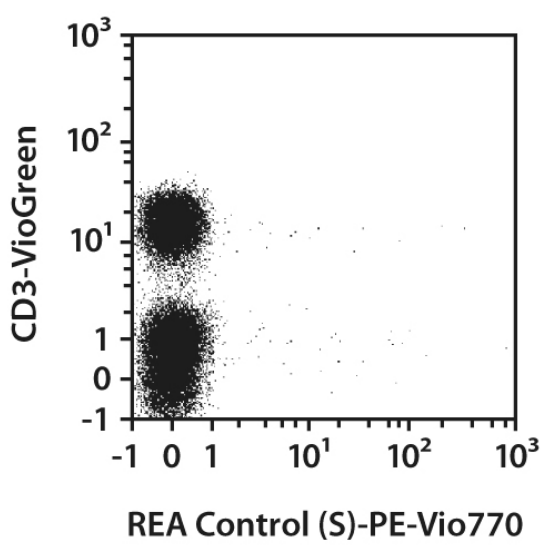

CD3-VioGreen
CD44-PE

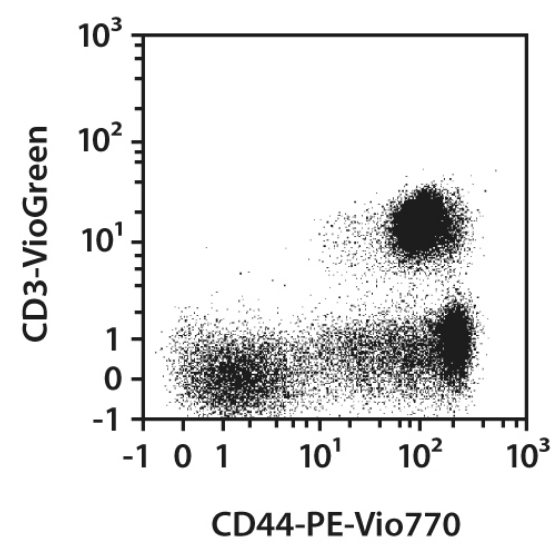

CD3-VioGreen
REA Control (S)-APC

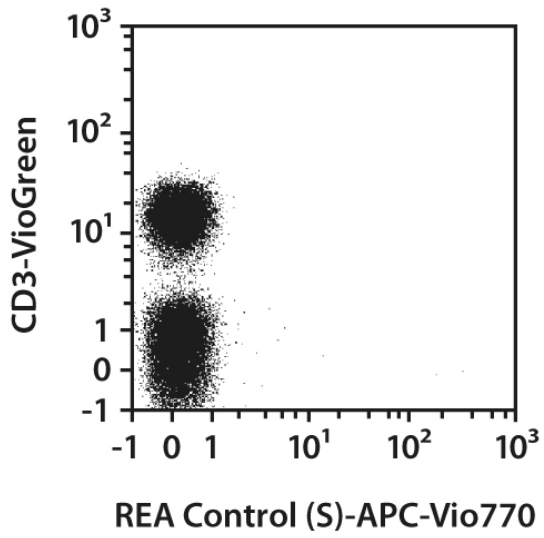

CD3-VioGreen
CD44-APC

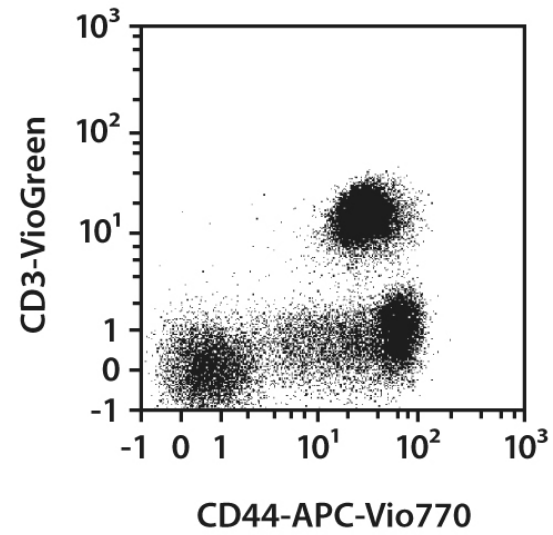

CD3-VioGreen
REA Control (S)-VioBlue

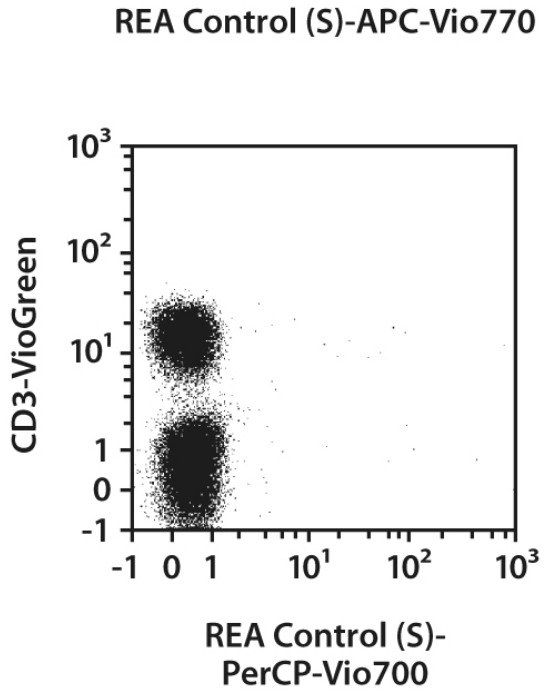

CD3-VioGreen
CD44-VioBlue

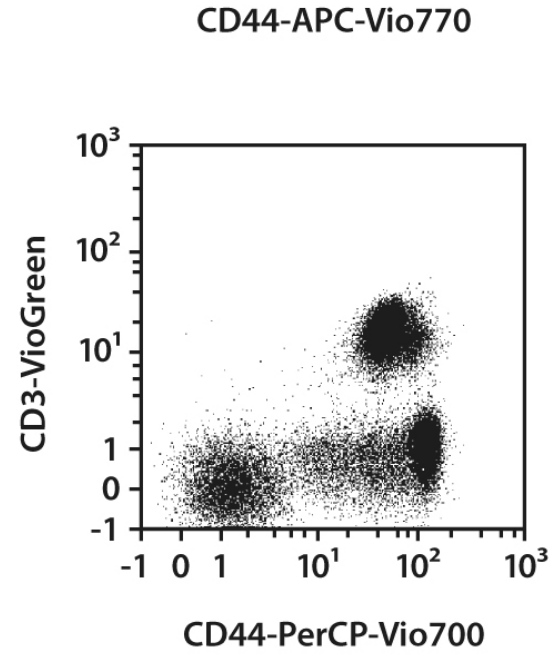

CD3-VioGreen
REA Control (S)-PE-Vio770

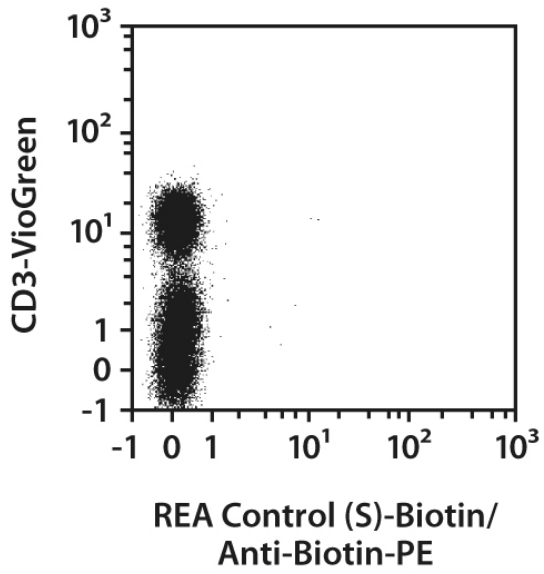

CD3-VioGreen
CD44-PE-Vio770

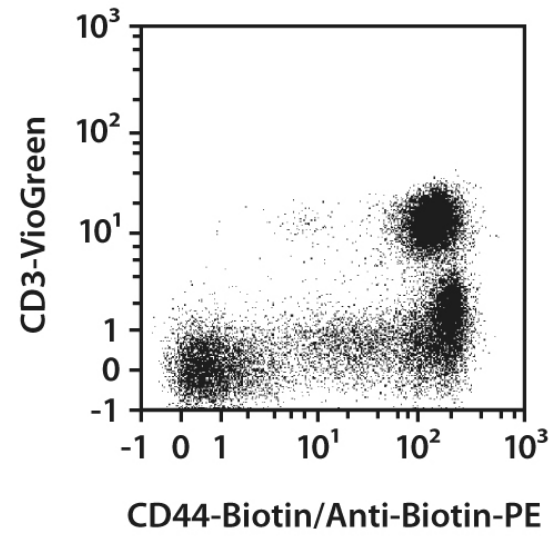












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