REA Control (S) antibodies

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.
REA Control (S)-APC	for 100 tests	130-113-434
REA Control (S)-FITC	for 100 tests	130-113-437
REA Control (S)-VioBright FITC	for 100 tests	130-113-443
REA Control (S)-PE	for 100 tests	130-113-438
REA Control (S)-VioBlue	for 100 tests	130-113-442
REA Control (S)-VioGreen	for 100 tests	130-113-444
REA Control (S)-PE-Vio615	for 100 tests	130-113-439
REA Control (S)-PE-Vio770	for 100 tests	130-113-440
REA Control (S)-APC-Vio770	for 100 tests	130-113-435
REA Control (S)-PerCP-Vio700	for 100 tests	130-113-441
REA Control (S)-VioBright 515	for 100 tests	130-113-445
REA Control (S)-Biotin	for 100 tests	130-113-436
REA Control (S)-VioBright 667	for 100 tests	130-118-217

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	KLH
Clone	REA293
Isotype	recombinant human IgG1
Alternative names of antigen	keyhole limpet hemocyanin
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze.

The REA Control (S) antibody clone REA293 is a universal isotype control that can be used with all recombinant engineered antibodies (REAfinity™ Antibodies) that recognize cell surface antigens. REAfinity Antibodies have been engineered for their high specificity and contain human IgG1 parts for constant regions. Although REAfinity Antibodies show virtually no binding to Fc receptors, the use of the clone REA293 is still recommended to control for other non Fc receptor–mediated non-specific binding of fluorochrome-conjugated REAfinity Antibodies to cells. Unspecific interactions of the fluorochrome with the cell surface can also be controlled for with conjugated versions of clone REA293.

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.

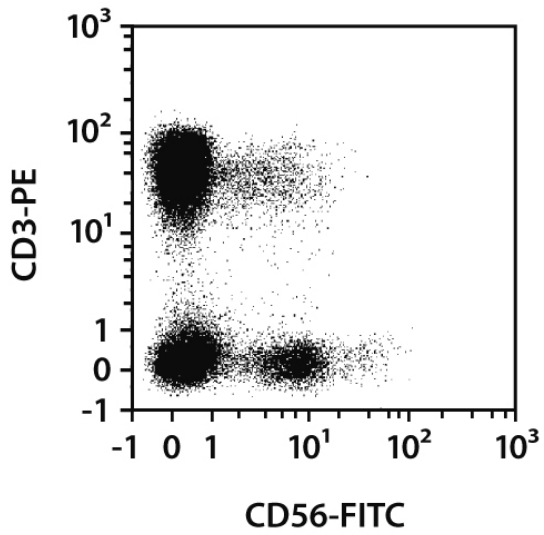
Protocol for cell surface staining

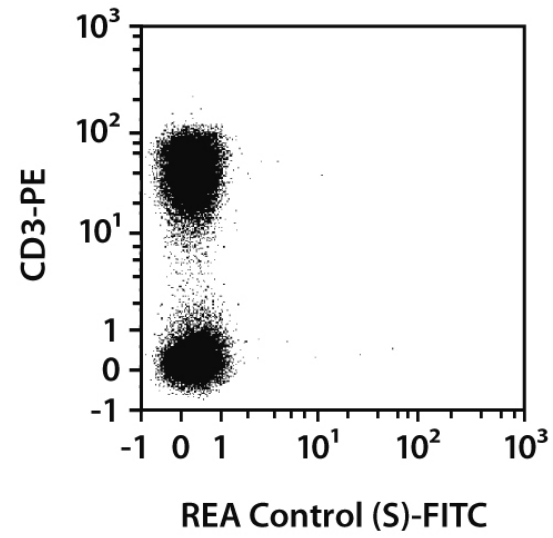
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell 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 REA Control (S) antibodies or with the corresponding CD56 (clone REA196) antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed with the MACSQuant®Analyzer. 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-PE
CD56-FITC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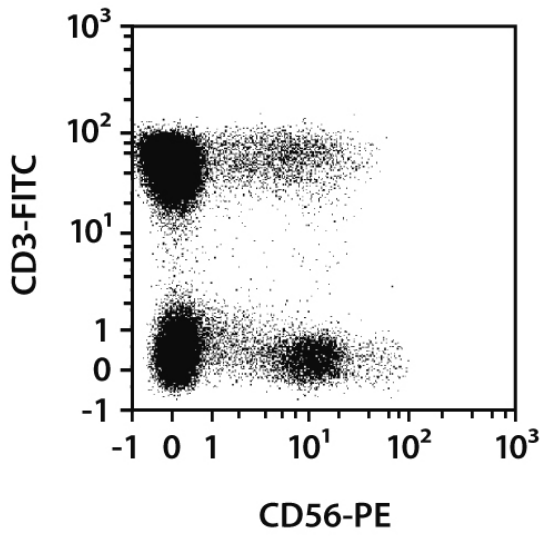
REA Control (S)-FITC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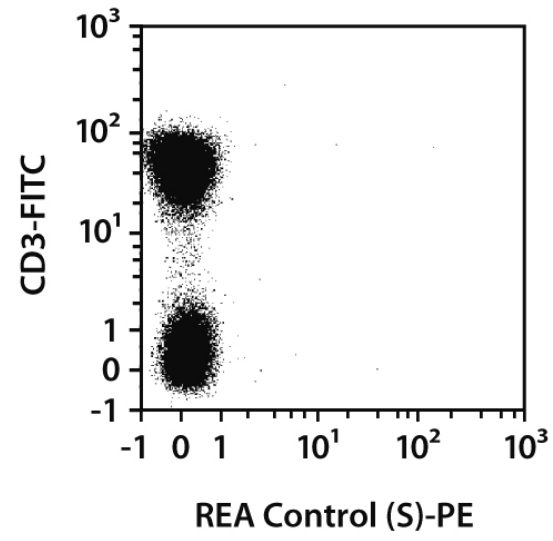
CD56-PE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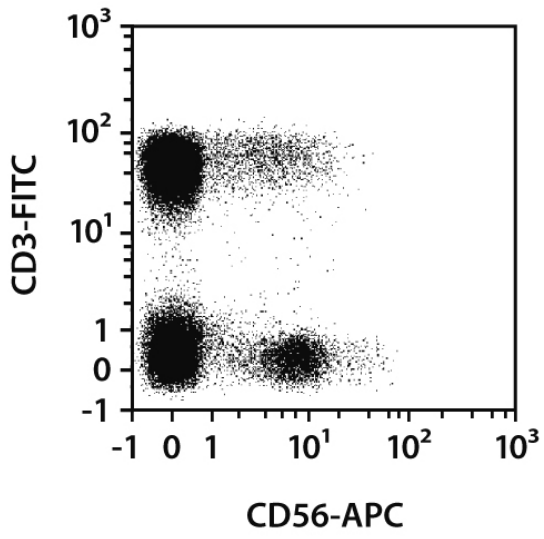
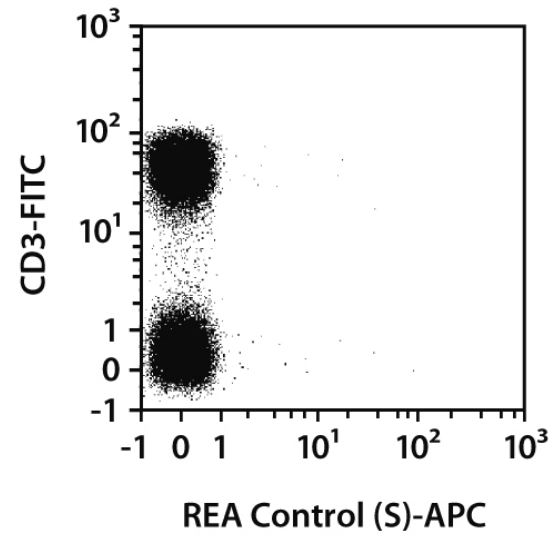
REA Control (S)-PE


CD3-FITC
CD56-APC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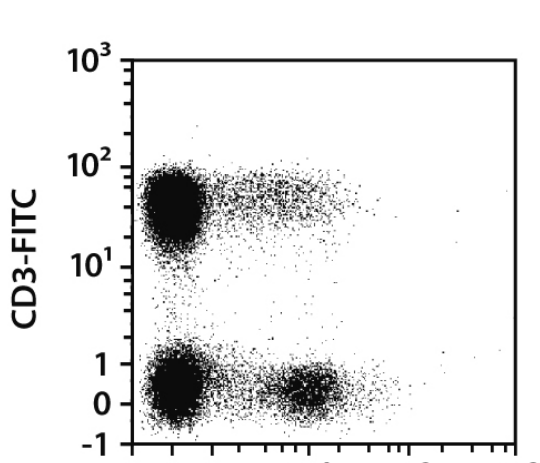
REA Control (S)-APC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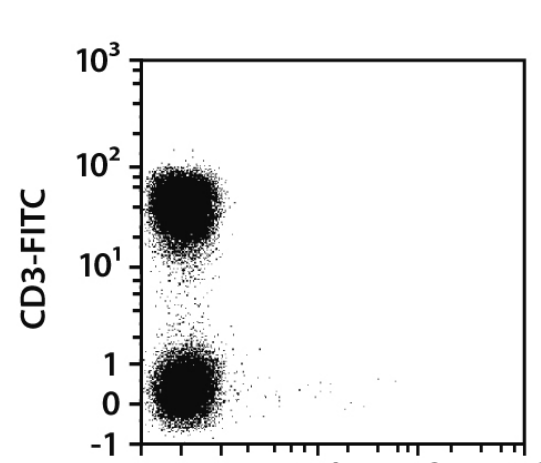
CD56-PE-Vio770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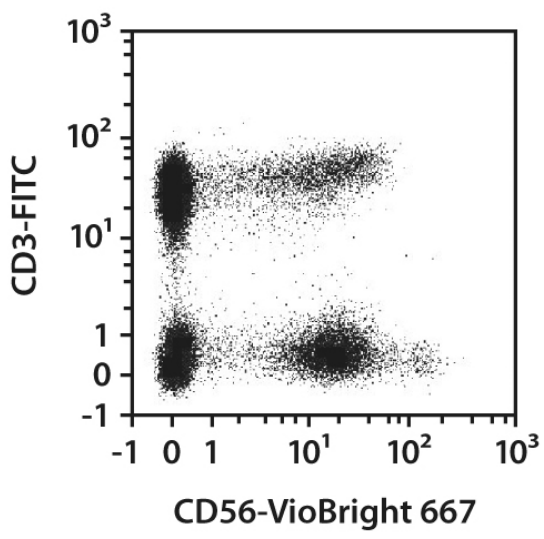
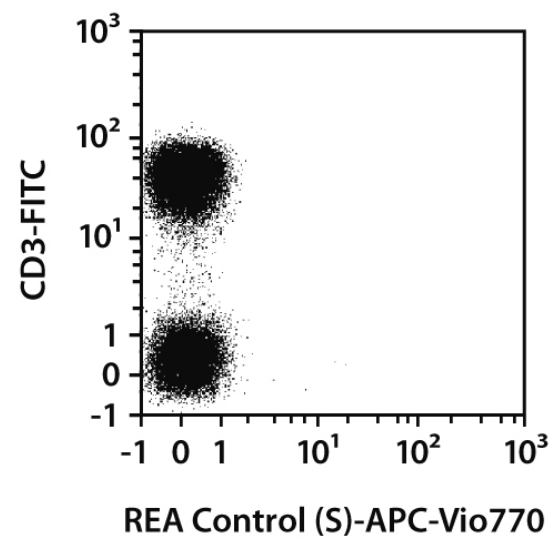
REA Control (S)-PE-Vio770


CD3-FITC
CD56-VioBright 667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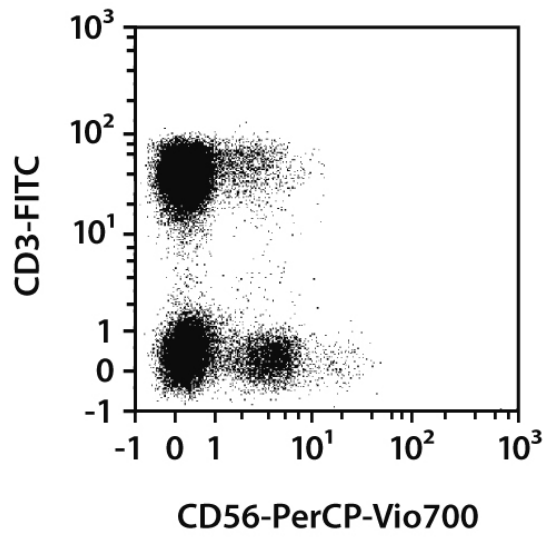
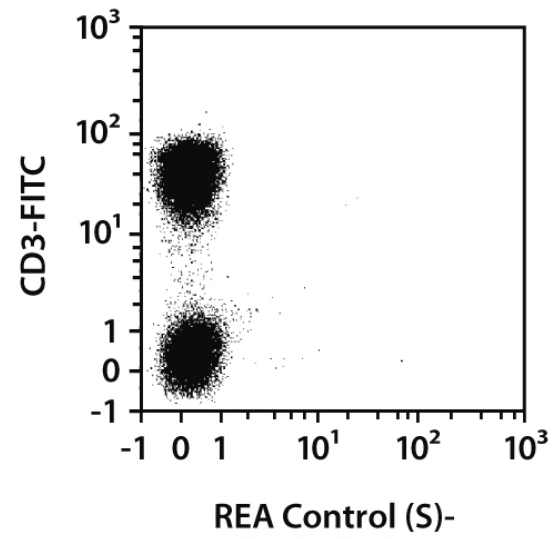
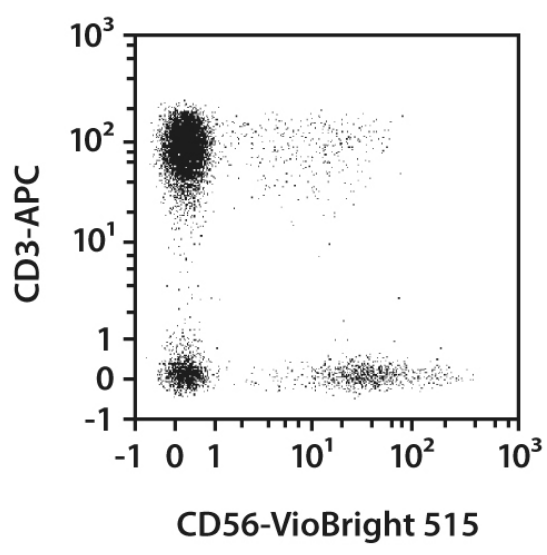
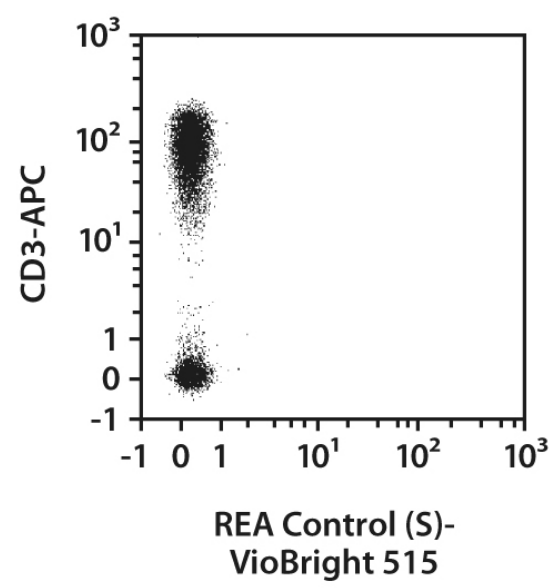
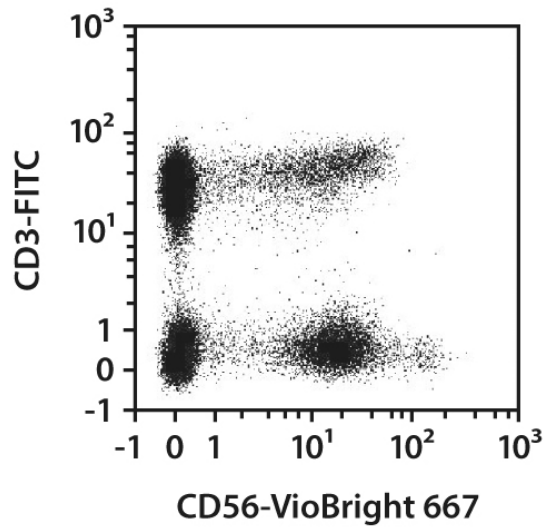
REA Control (S)-APC-Vio770

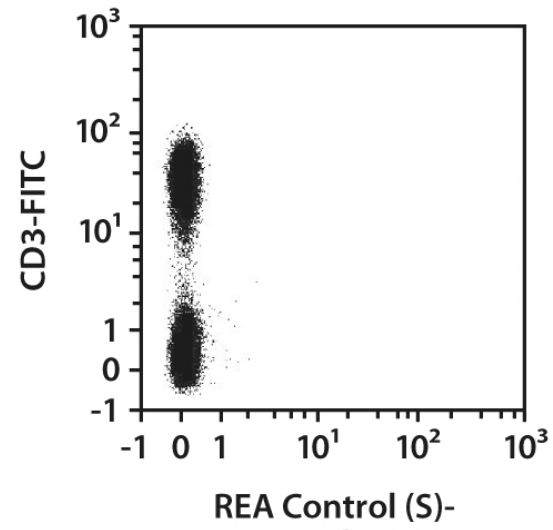


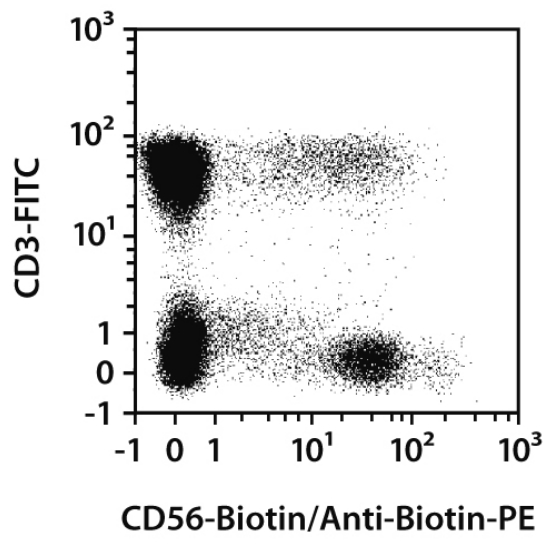


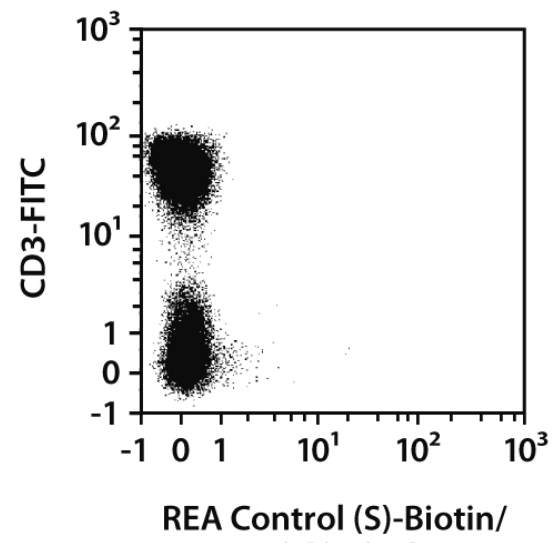












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