CD4 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.
CD4-APC	for 30 tests	130-113-784
CD4-FITC	for 30 tests	130-114-722
CD4-FITC	for 100 tests	130-114-531
CD4-FITC	for 500 tests	130-114-585
CD4-VioBright FITC	for 30 tests	130-113-791
CD4-VioBright FITC	for 100 tests	130-113-229
CD4-PE	for 30 tests	130-113-787
CD4-PE	for 100 tests	130-113-225
CD4-APC	for 100 tests	130-113-222
CD4-VioBlue	for 30 tests	130-114-725
CD4-VioBlue	for 100 tests	130-114-534
CD4-VioGreen	for 30 tests	130-113-792
CD4-VioGreen	for 100 tests	130-113-230
CD4-PE-Vio615	for 30 tests	130-113-788
CD4-PE-Vio615	for 100 tests	130-113-226
CD4-PE-Vio770	for 30 tests	130-113-789
CD4-PE-Vio770	for 100 tests	130-113-227
CD4-APC-Vio770	for 30 tests	130-113-785
CD4-APC-Vio770	for 100 tests	130-113-223
CD4-PerCP-Vio700	for 30 tests	130-113-790
CD4-PerCP-Vio700	for 100 tests	130-113-228
CD4-Vio515	for 30 tests	130-115-298
CD4-Vio515	for 100 tests	130-115-199
CD4-VioBright 515	for 30 tests	130-114-726
CD4-VioBright 515	for 100 tests	130-114-535
CD4-Vio667	for 30 tests	130-115-299
CD4-Vio667	for 100 tests	130-115-200
CD4-VioBright 667	for 30 tests	130-114-723
CD4-VioBright 667	for 100 tests	130-114-532
CD4-Biotin	for 30 tests	130-113-786
CD4-Biotin	for 100 tests	130-113-224

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	REA623
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	T4, Leu-3, CD4mut
Entrez Gene ID	920
Molecular mass of antigen [kDa]	48
Distribution of antigen	monocytes, T cells, thymocytes, T helper cells
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.

Clone REA623 recognizes the human CD4 antigen, a 55 kDa single-pass type I membrane protein, also known as T4/Leu-3. CD4 is highly expressed on T helper cells and at a lower level on monocytes and dendritic cells. It is involved in the recognition of MHC class II/peptide complexes by the TCR heterodimers and is the receptor for the human immunodeficiency virus (HIV). Additional information: Clone REA623 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

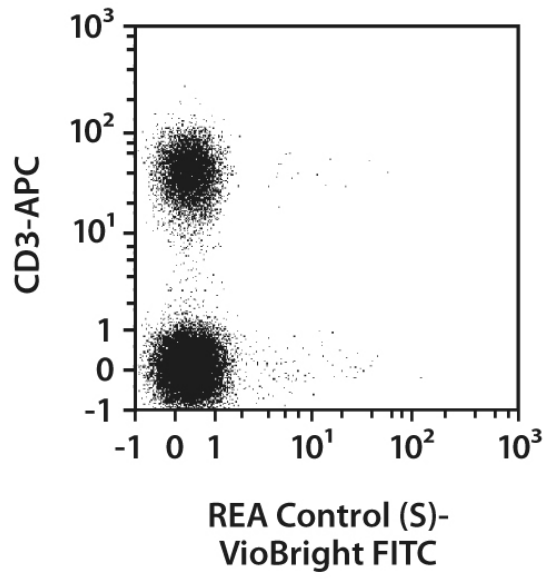
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

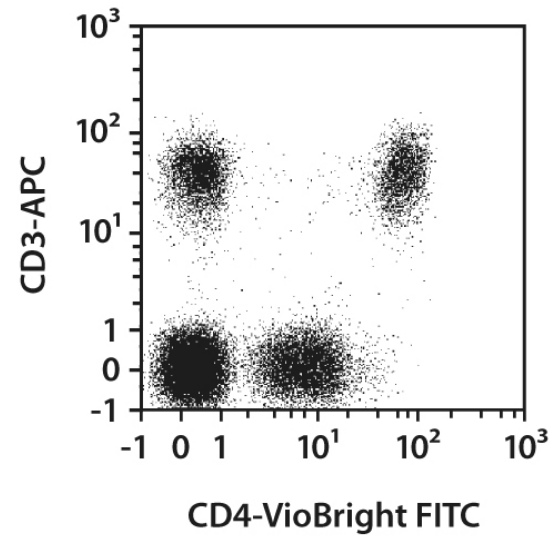
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

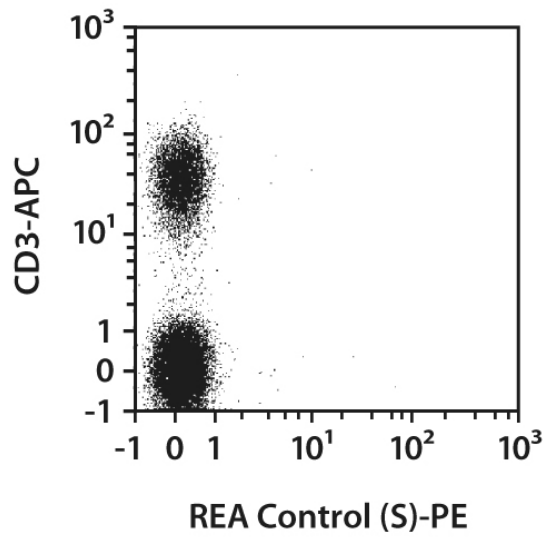
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

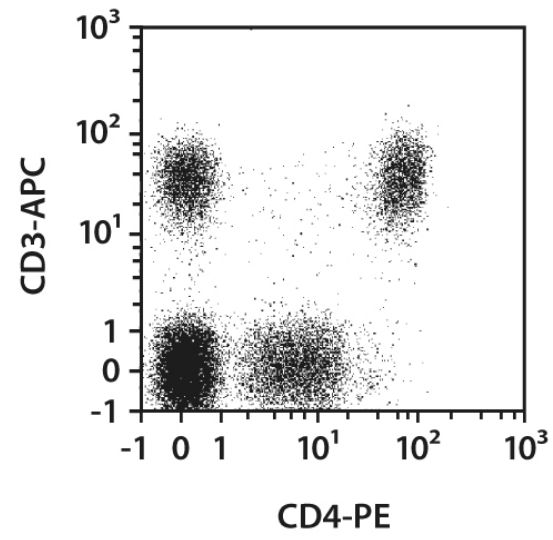
Examples of immunofluorescent staining

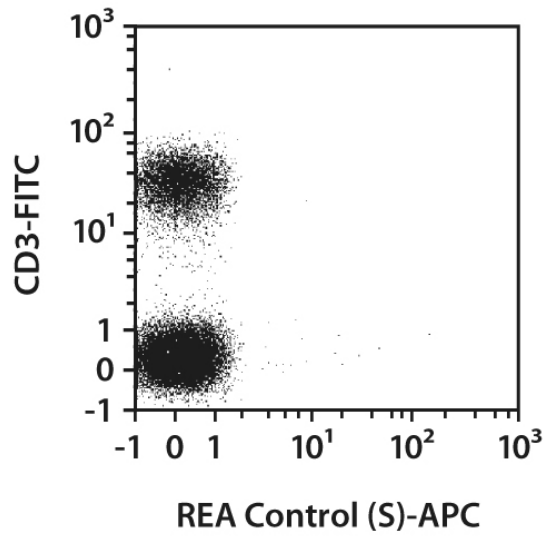
Human peripheral blood mononuclear cells (PBMCs) were stained with CD4 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD3 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding 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.

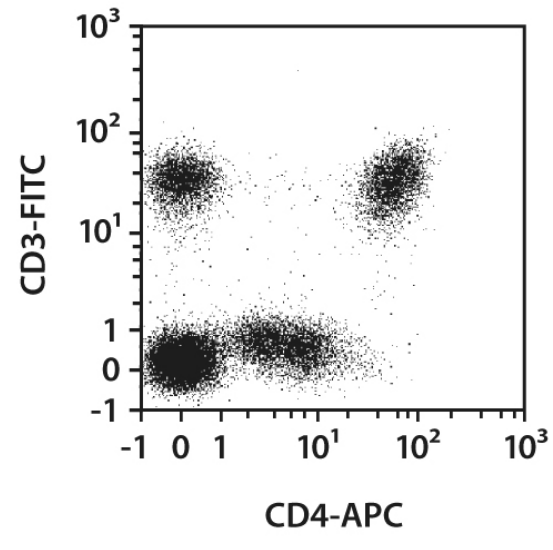


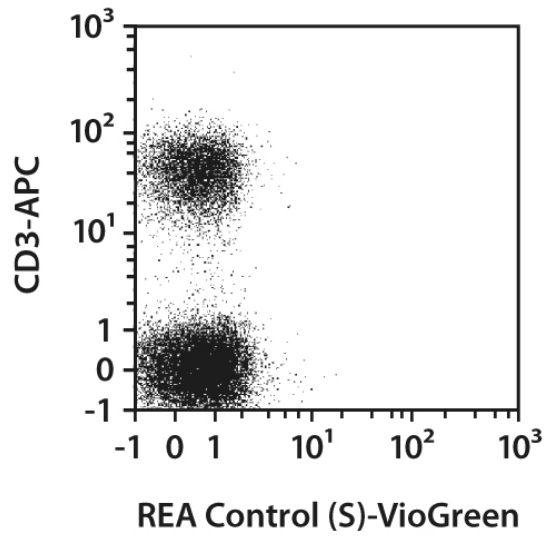


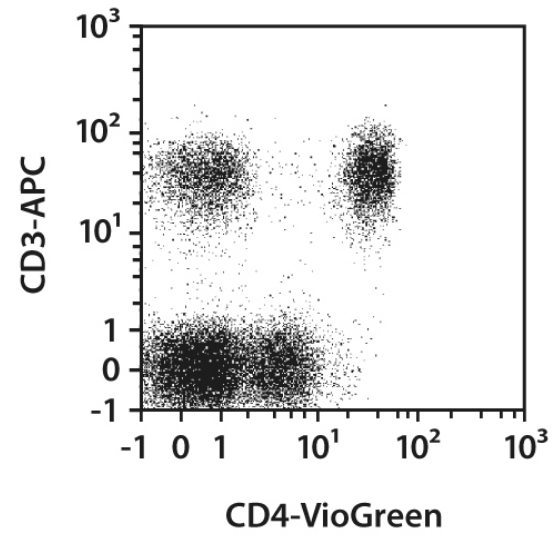


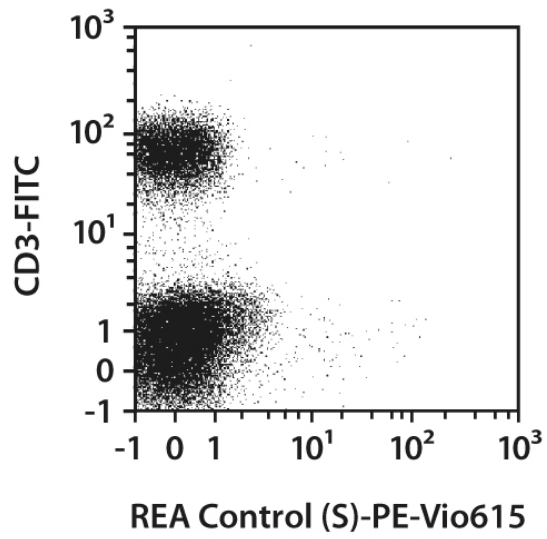








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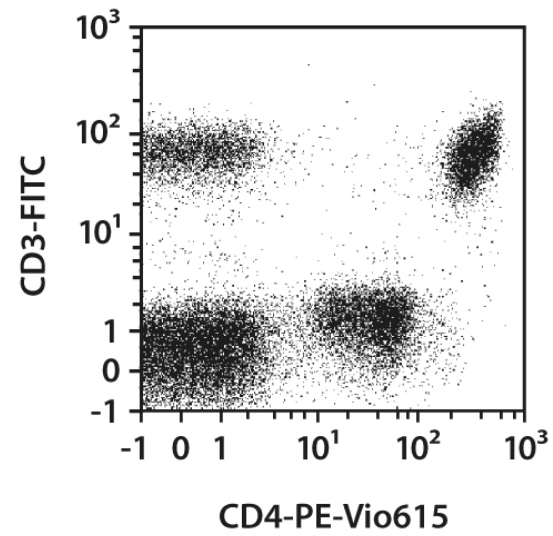
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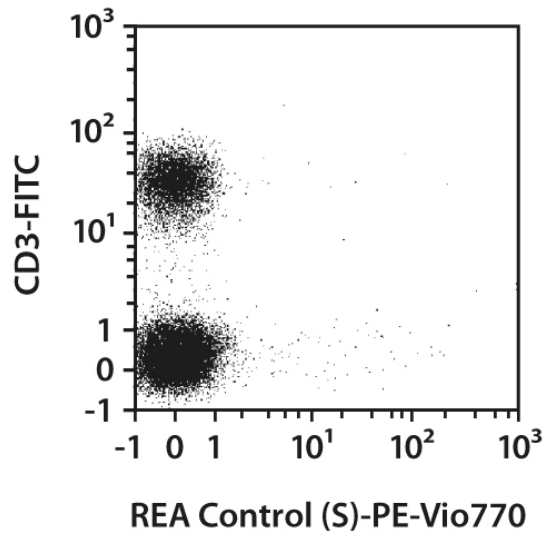
CD3-FITC
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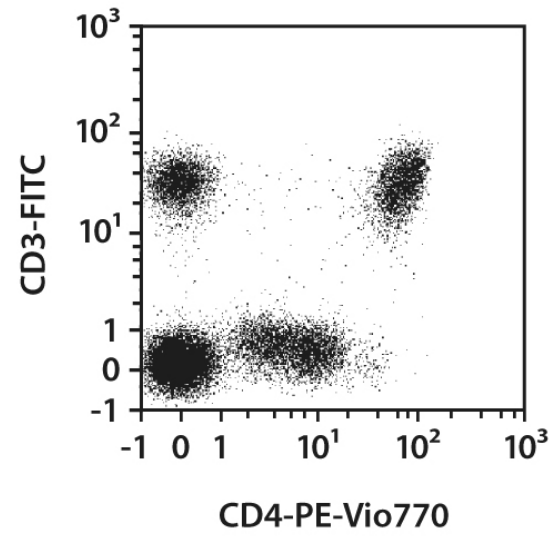
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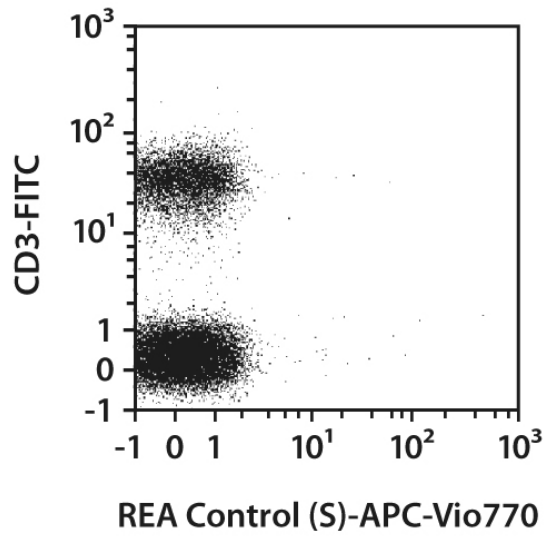
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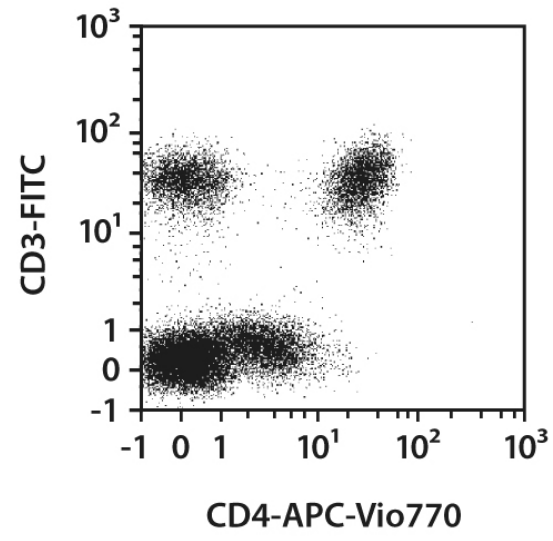
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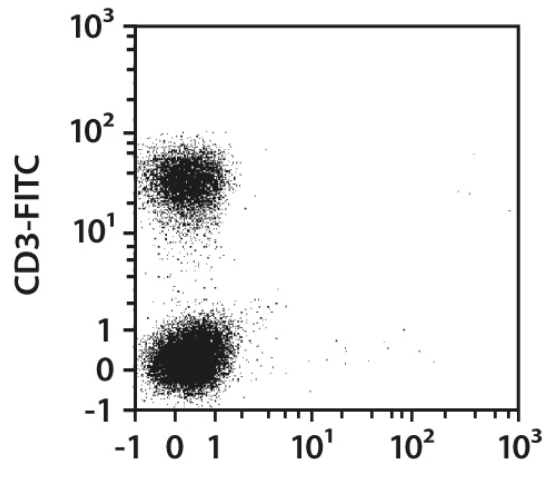
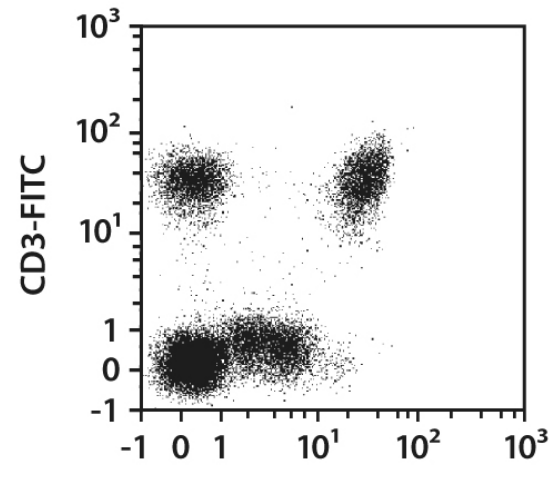

CD3-FITC
REA Control (S)-
PerCP-Vio700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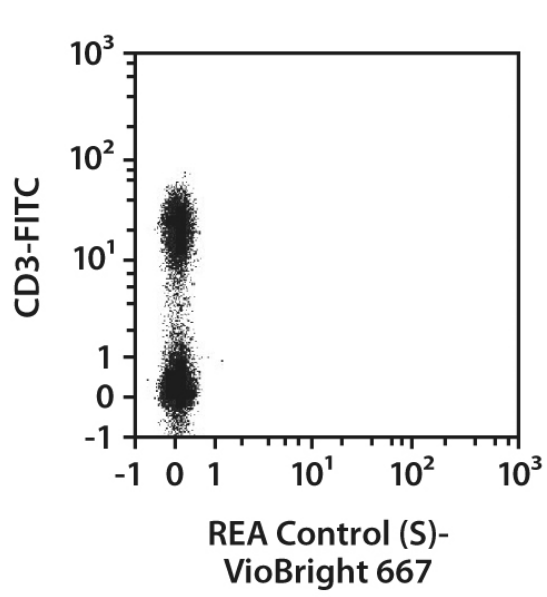
CD4-PerCP-Vio700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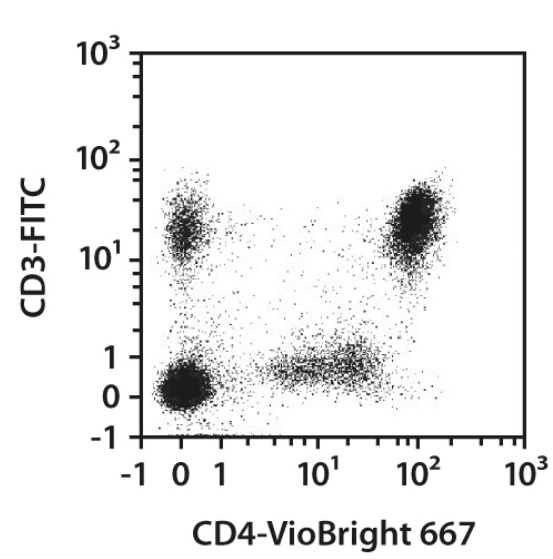
VioBright 667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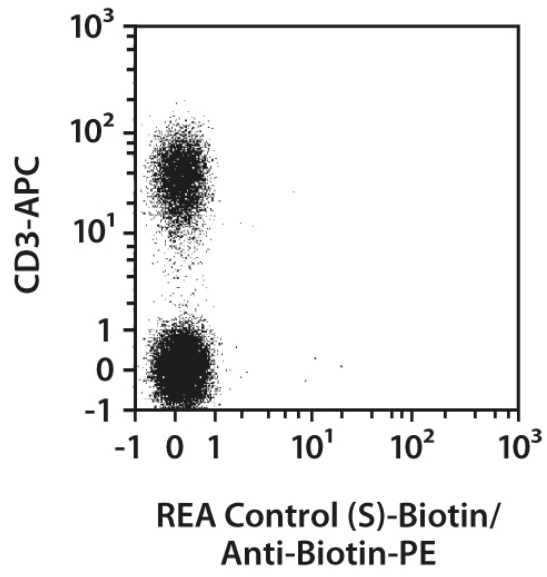

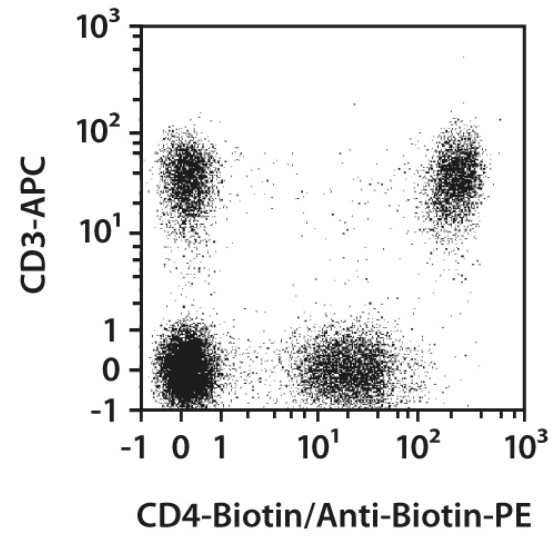

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