# CD3 antibodies, non-human primate

## For research use only

6
One test corresponds to labeling of up to 10  cells in a total volume of 100 µL

| Product | Content | Order no. |
|---|---|---|
| CD3-Biotin | for 100 tests | 130-116-624 |
| CD3-FITC | for 30 tests | 130-116-746 |
| CD3-FITC | for 100 tests | 130-116-625 |
| CD3-PE | for 30 tests | 130-116-747 |
| CD3-PE | for 100 tests | 130-116-626 |
| CD3-APC | for 30 tests | 130-116-748 |
| CD3-APC | for 100 tests | 130-116-627 |
| CD3-VioBlue | for 30 tests | 130-116-753 |
| CD3-VioBlue | for 100 tests | 130-116-632 |
| CD3-VioGreen | for 30 tests | 130-116-754 |
| CD3-VioGreen | for 100 tests | 130-116-633 |
| CD3-PE-Vio615 | for 30 tests | 130-116-755 |
| CD3-PE-Vio615 | for 100 tests | 130-116-634 |
| CD3-PE-Vio770 | for 30 tests | 130-116-749 |
| CD3-PE-Vio770 | for 100 tests | 130-116-628 |
| CD3-APC-Vio770 | for 100 tests | 130-116-629 |
| CD3-PerCP-Vio700 | for 30 tests | 130-116-751 |
| CD3-PerCP-Vio700 | for 100 tests | 130-116-630 |
| CD3-VioBright 515 | for 30 tests | 130-116-752 |
| CD3-VioBright 515 | for 100 tests | 130-116-631 |
| CD3-Biotin | for 30 tests | 130-116-745 |

## 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** | REA994 |
| **Isotype** | recombinant human IgG1 |
| **Isotype control** | REA Control (S) antibodies |

| | |
|---|---|
| **Alternative names of antigen** | T3 |
| **Entrez Gene ID** | 699582, 705270, 697814, 699467 |
| **Cross-reactivity** | pigtail monkey ( *Macaca nemestrina* ), olive baboon ( *Papio anubis* ), african green monkey (*Chlorocebus aethiops*) |
| **Distribution of antigen** | T cells, NKT cells, NK 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 REA994 recognizes rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) CD3 T cells. The antibody has been reported to be cross-reactive with pigtail monkey (*Macaca nemestrina*), olive baboon monkey (*Papio anubis* ), and african green monkey (*Chlorocebus aethiops*) T cells. CD3 is expressed on all T cells and on a subset of NK cells. It is associated with the T cell receptor (TCR) and is responsible for its signal transduction. Additional information: Clone REA994 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.

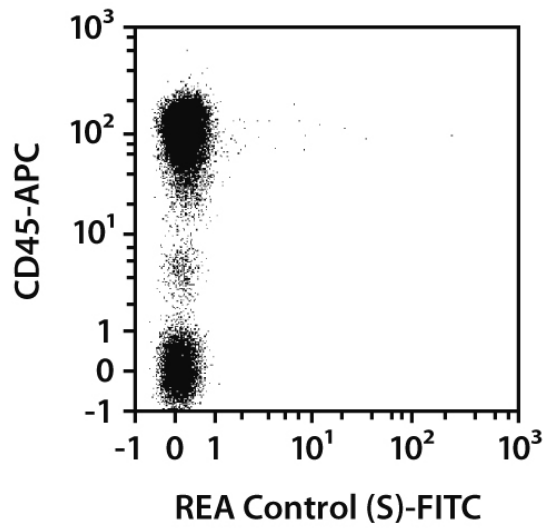
## Protocol for cell surface staining

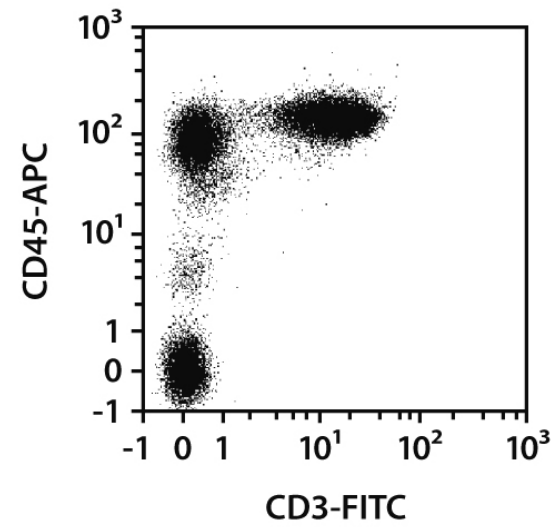
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to $10^6$ cells/100 µL.
- Volumes given below are for up to $10^6$ nucleated cells. When working with fewer than $10^6$ cells, use the same volumes as indicated. When working with higher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

Peripheral blood mononuclear cells (PBMCs) from rhesus monkeys were stained with CD3 antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD45 antibodies and analyzed by flow cytometry using 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.


CD45-APC
REA Control (S)-FITC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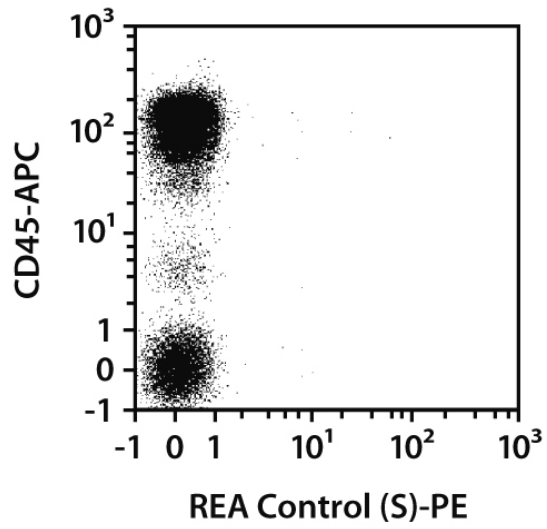
CD3-FITC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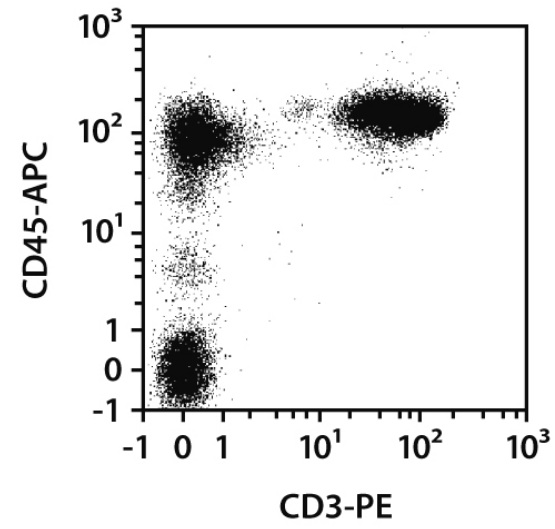
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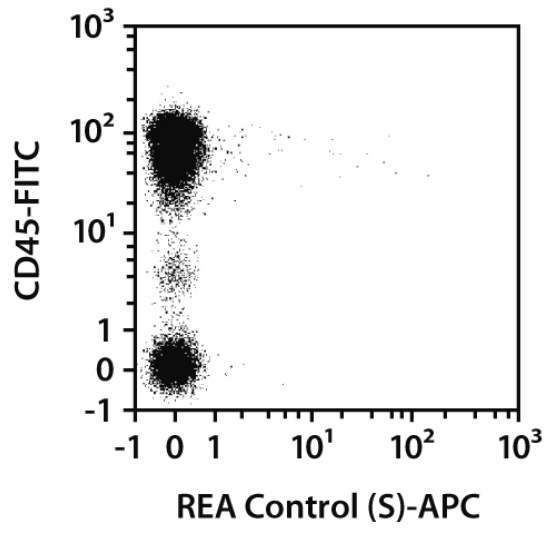
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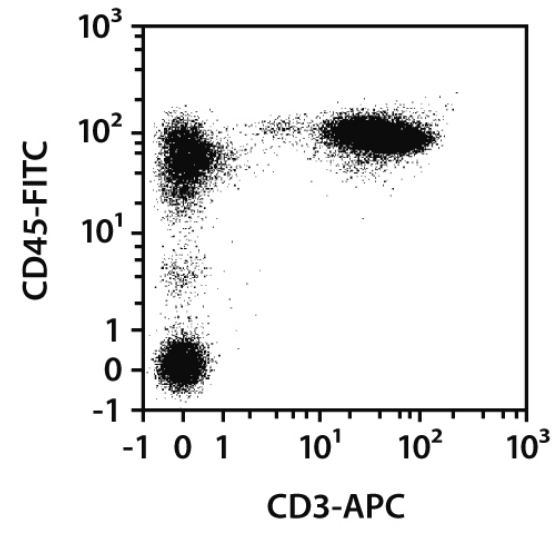
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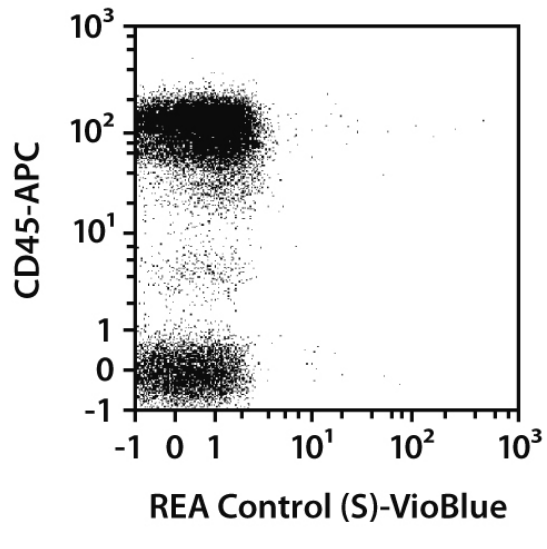
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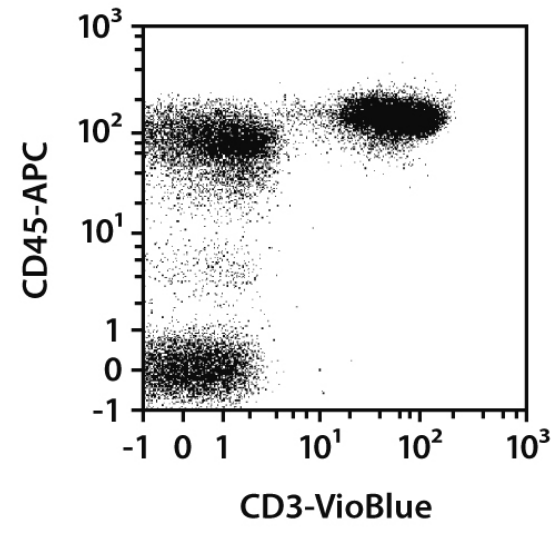
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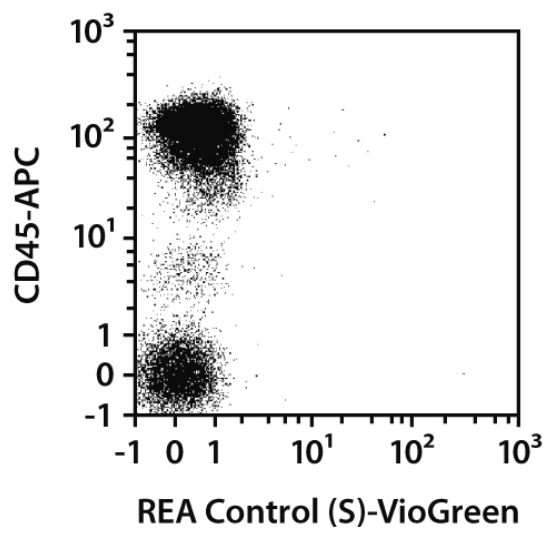
CD3-VioBlue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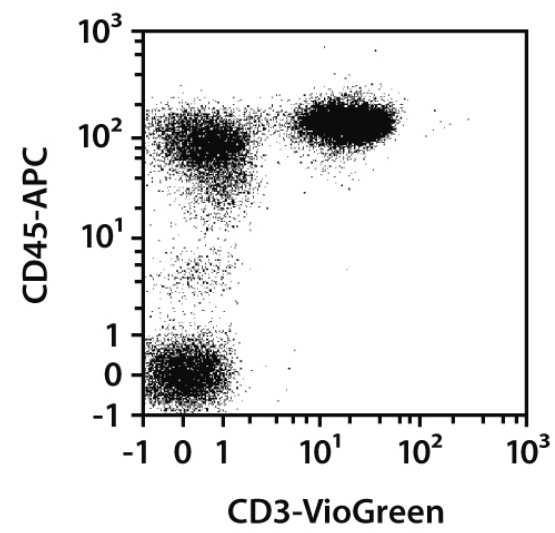
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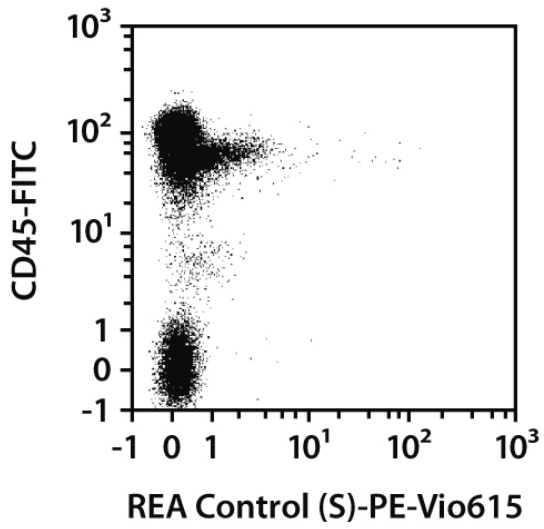
CD3-VioGreen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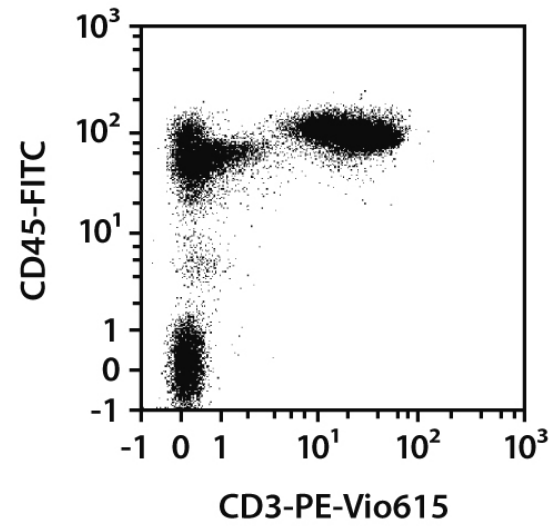
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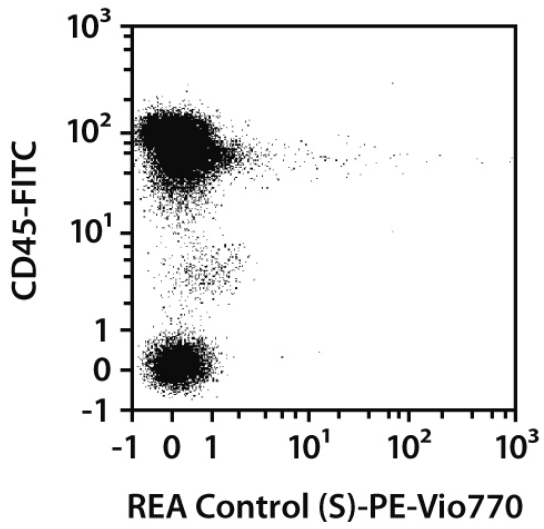
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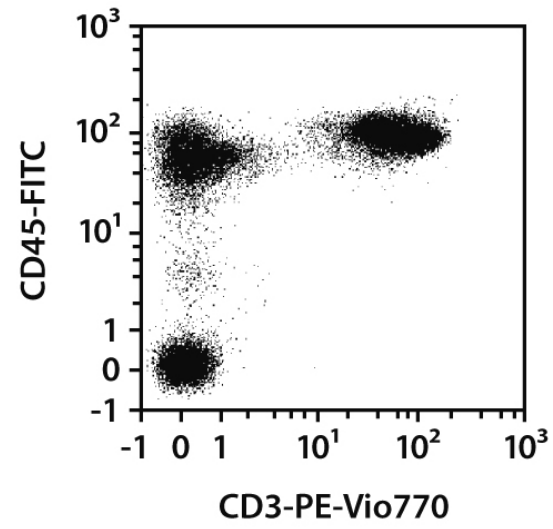
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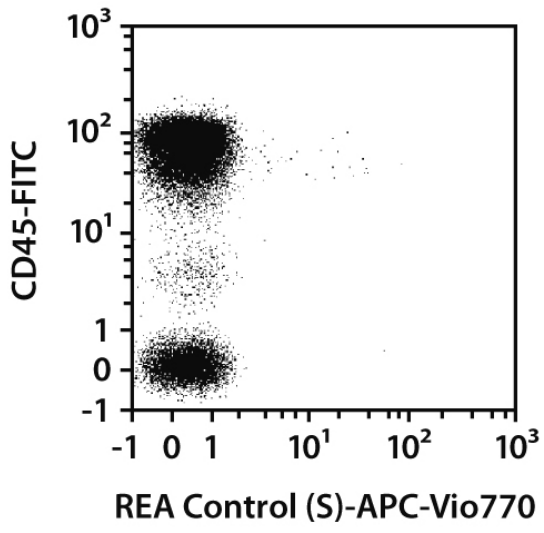
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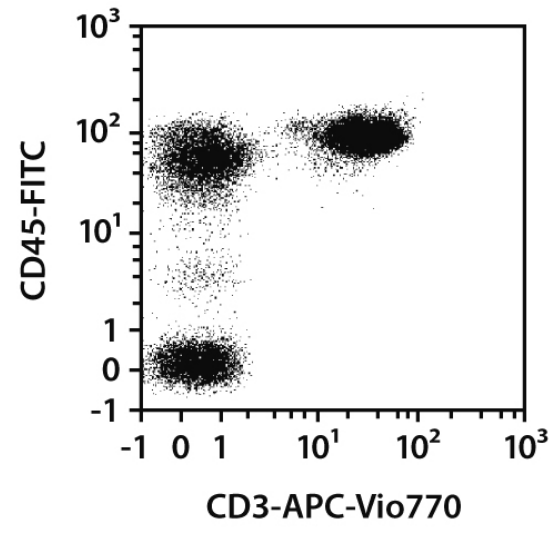
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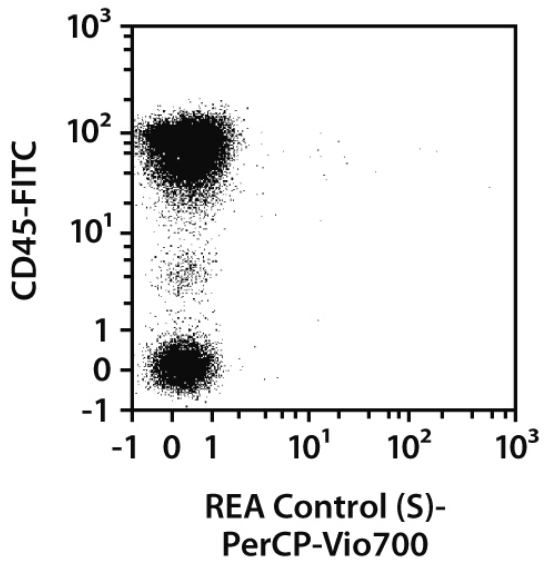
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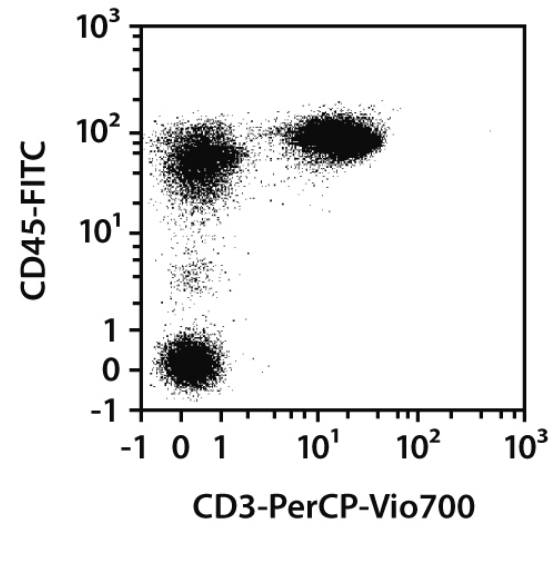
PerCP-Vio700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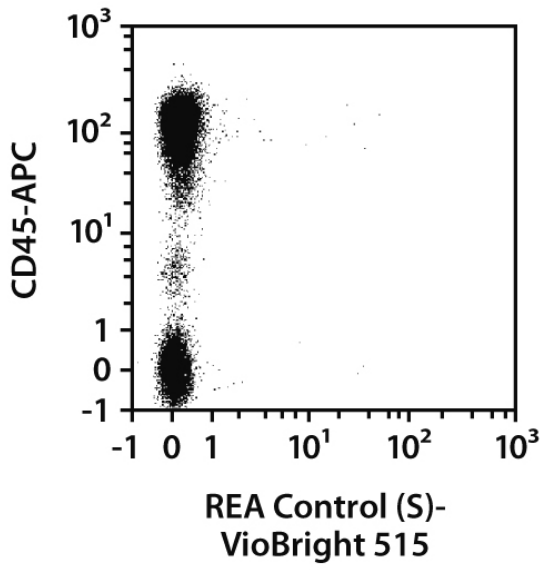
CD3-PerCP-Vio700


CD45-APC
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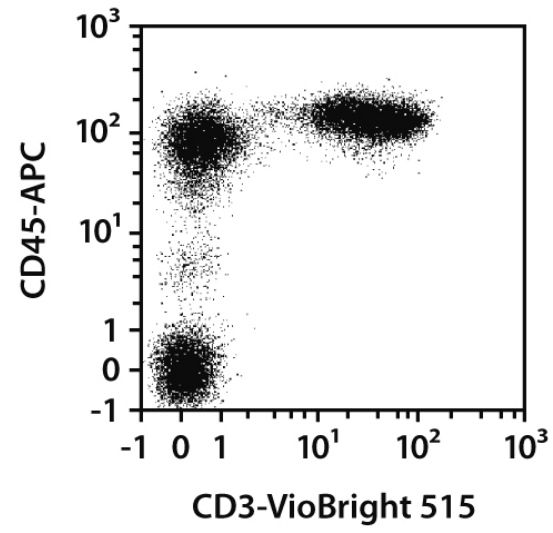
VioBright 515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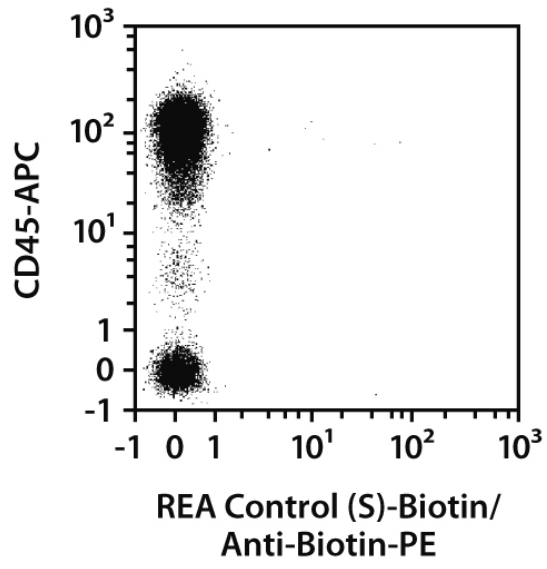


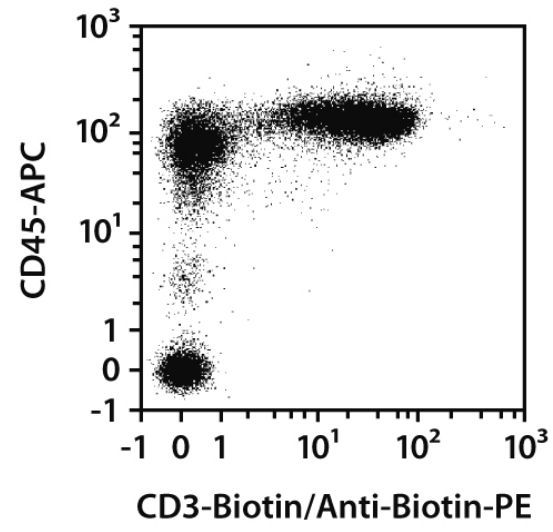


## Warranty

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