CD33 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.
CD33-VioGreen	for 100 tests	130-111-025
CD33-FITC	for 30 tests	130-111-135
CD33-FITC	for 100 tests	130-111-018
CD33-PE	for 30 tests	130-111-136
CD33-PE	for 100 tests	130-111-019
CD33-APC	for 30 tests	130-111-137
CD33-APC	for 100 tests	130-111-020
CD33-VioBlue	for 30 tests	130-111-141
CD33-VioBlue	for 100 tests	130-111-024
CD33-VioGreen	for 30 tests	130-111-142
CD33-PE-Vio615	for 30 tests	130-111-143
CD33-PE-Vio615	for 100 tests	130-111-026
CD33-PE-Vio770	for 30 tests	130-111-138
CD33-PE-Vio770	for 100 tests	130-111-021
CD33-APC-Vio770	for 30 tests	130-111-139
CD33-APC-Vio770	for 100 tests	130-111-022
CD33-PerCP-Vio700	for 30 tests	130-111-140
CD33-PerCP-Vio700	for 100 tests	130-111-023
CD33-VioBright 515	for 30 tests	130-111-144
CD33-VioBright 515	for 100 tests	130-111-027
CD33-Biotin	for 30 tests	130-111-134
CD33-Biotin	for 100 tests	130-111-017

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	CD33
Clone	REA775
Isotype	recombinant human IgG1

Isotype control	REA Control (S) antibodies
Alternative names of antigen	Siglec-3, p67, My9
Entrez Gene ID	945
Molecular mass of antigen [kDa]	38
Cross-reactivity	cynomolgus monkey (*Macaca fascicularis*), rhesus monkey (*Macaca mulatta*)
Distribution of antigen	basophils, dendritic cells, granulocytes, Langerhans cells, leukemia cells, macrophages, mast cells, monocytes, myeloid cells, NK cells, 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 REA775 recognizes the human CD33 antigen, a 67 kDa glycoprotein belonging to the sialoadhesin superfamily. The CD33 antigen is highly expressed on human monocytes but weakly on granulocytes and some – but not all – myeloid dendritic cells. The CD33 antigen is also found on myeloid progenitor cells (CFU-GEMM, CFU GM, CFU-G, BFU-E) but is not expressed on lymphocytes, platelets, erythrocytes, or primitive hematopoietic stem cells. Additional information: Clone REA775 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.

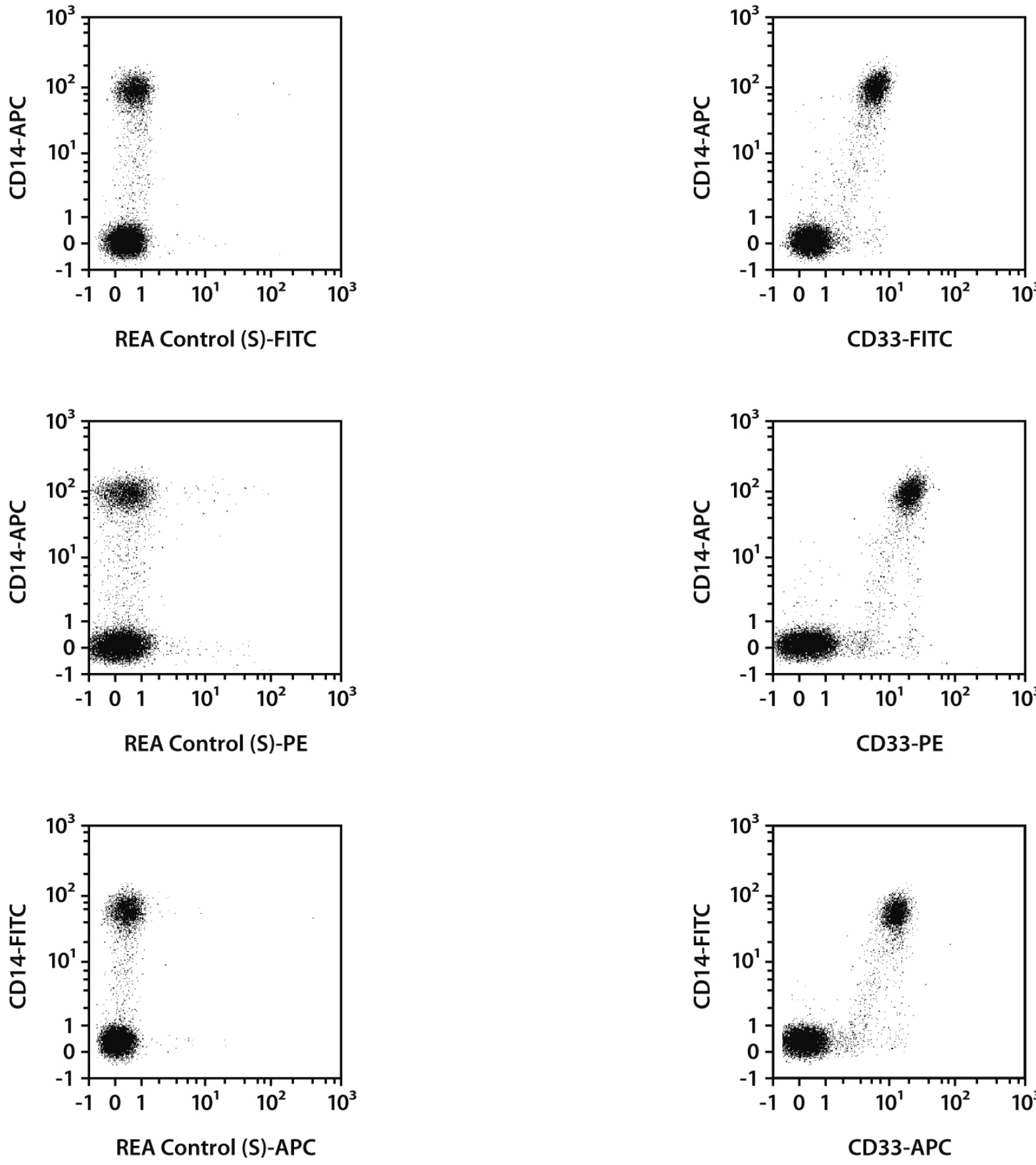
Protocol for cell surface staining

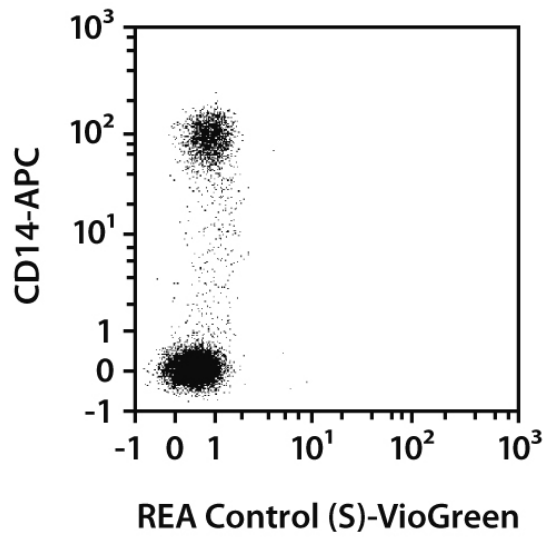
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

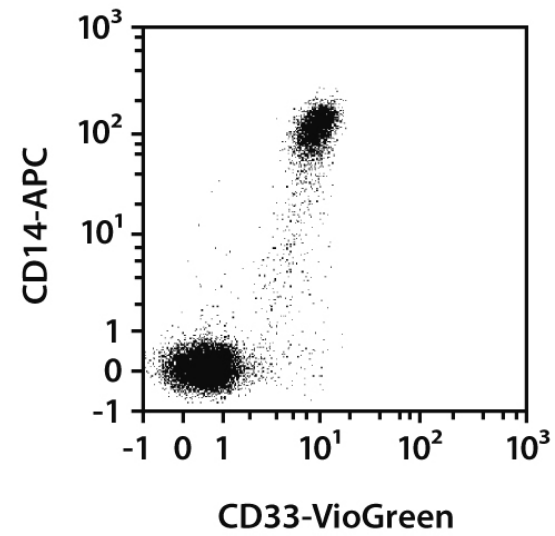
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

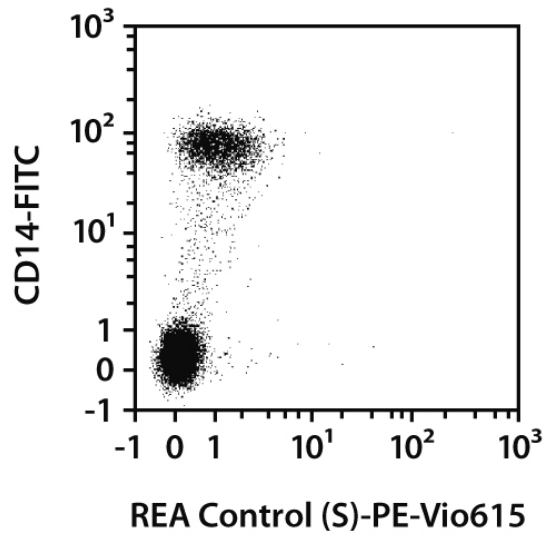
Examples of immunofluorescent staining

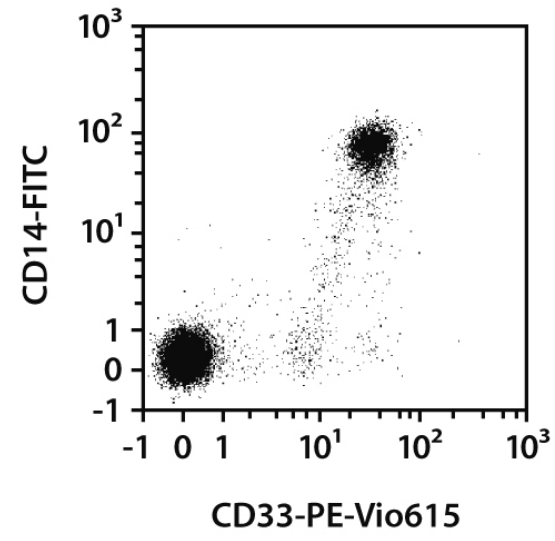
Human peripheral blood mononuclear cells (PBMCs) were stained with CD33 antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD14 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding 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.

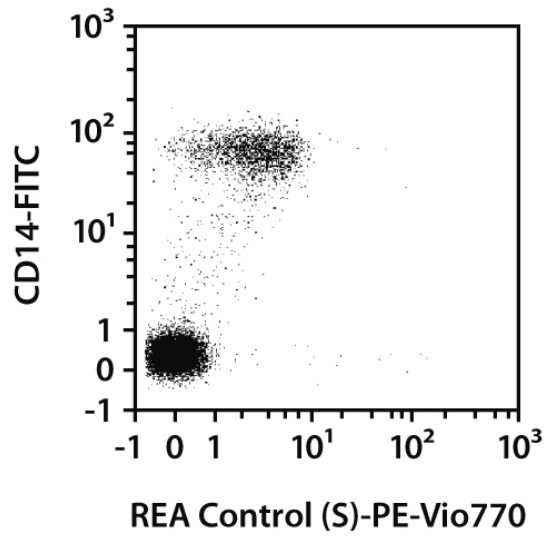


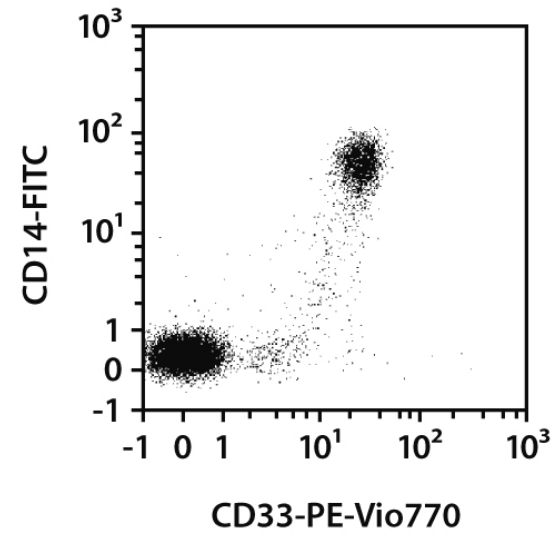

CD14-APC
REA Control (S)-VioGreen

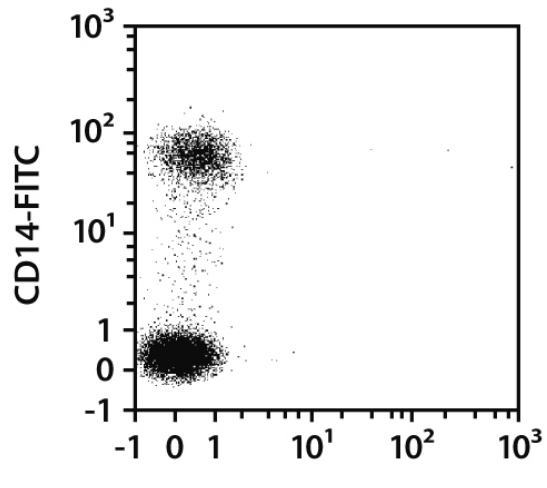

CD14-APC
CD33-VioGreen

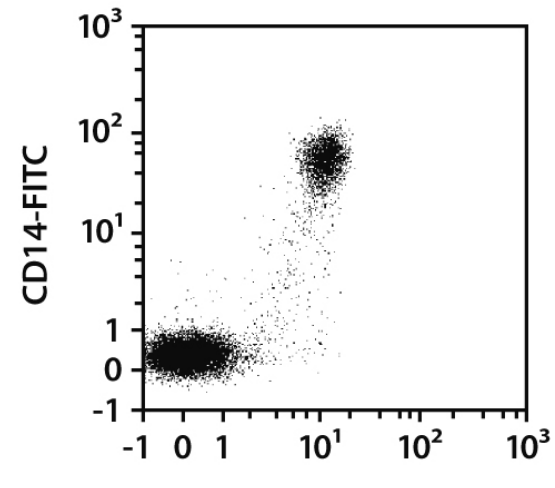

CD14-FITC
REA Control (S)-PE-Vio615

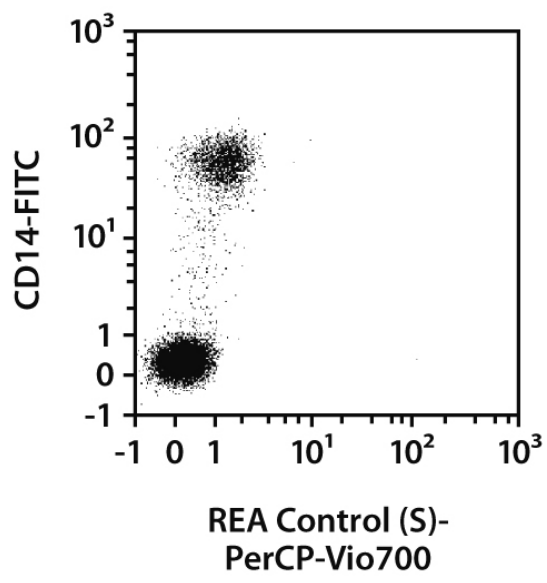

CD14-FITC
CD33-PE-Vio615

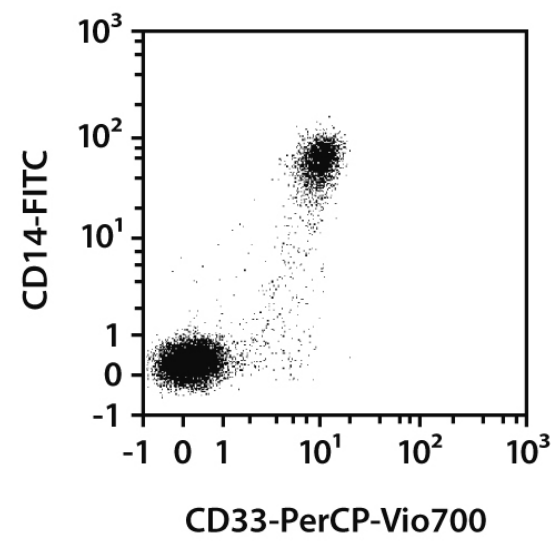

CD14-FITC
REA Control (S)-PE-Vio770

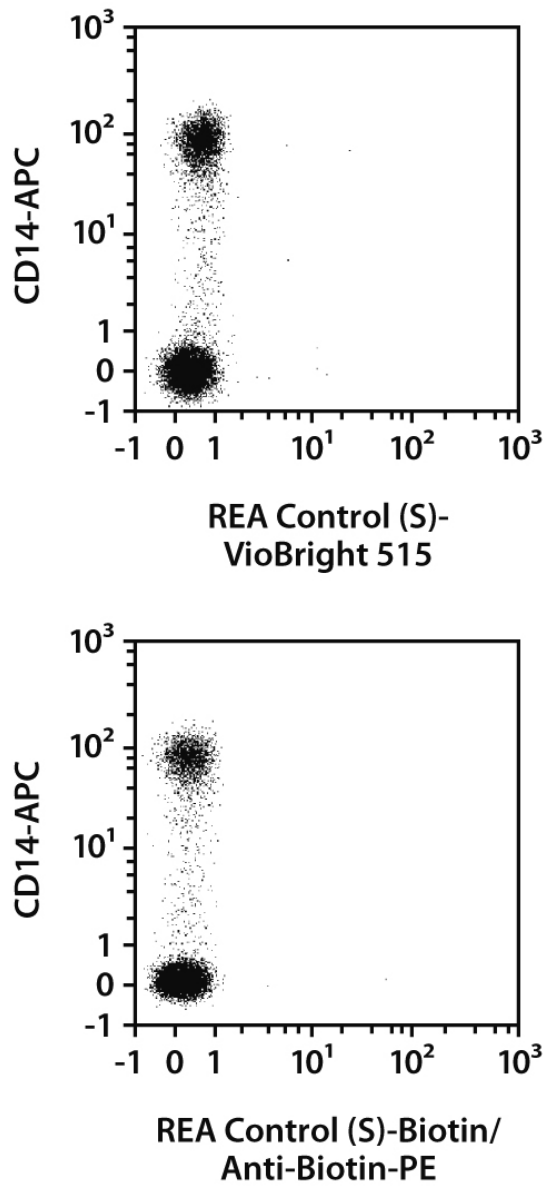

CD14-FITC
CD33-PE-Vio770

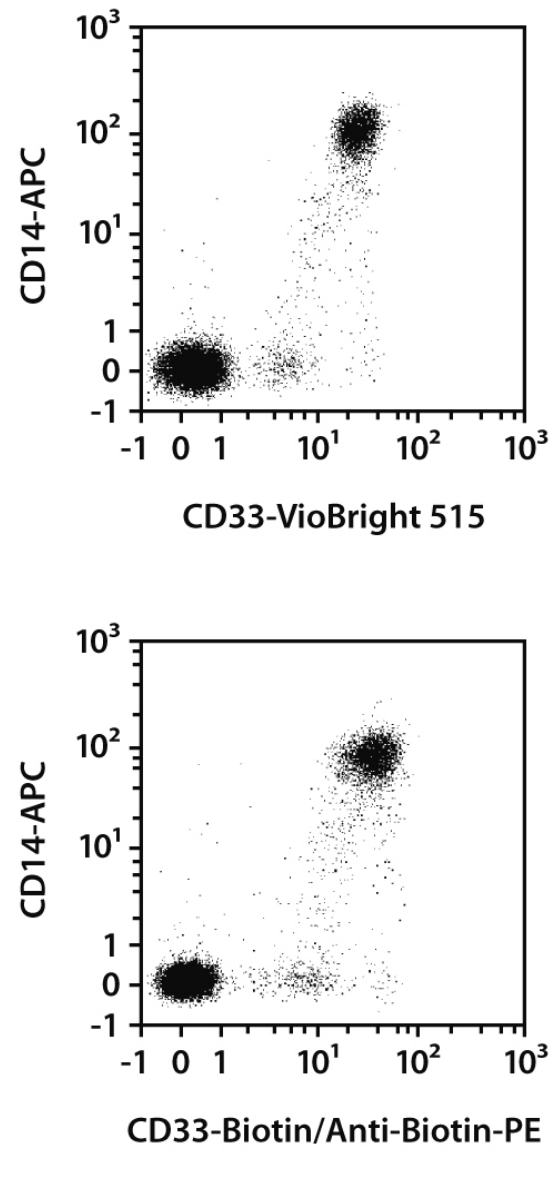

CD14-FITC
REA Control (S)-APC-Vio770


CD14-FITC
CD33-APC-Vio770


CD14-FITC
REA Control (S)-
PerCP-Vio700


CD14-FITC
CD33-PerCP-Vio700





Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.