

# CD20 antibodies, human

## For research use only

One test corresponds to labeling of up to  $10^6$  cells in a total volume of 100  $\mu$ L

Product	Content	Order no.
CD20-Biotin	for 100 tests	130-111-336
CD20-FITC	for 30 tests	130-111-523
CD20-FITC	for 100 tests	130-111-337
CD20-PE	for 30 tests	130-111-524
CD20-PE	for 100 tests	130-111-338
CD20-APC	for 30 tests	130-111-525
CD20-APC	for 100 tests	130-111-339
CD20-VioBlue	for 30 tests	130-111-531
CD20-VioBlue	for 100 tests	130-111-345
CD20-VioGreen	for 30 tests	130-111-532
CD20-VioGreen	for 100 tests	130-111-346
CD20-PE-Vio615	for 30 tests	130-111-533
CD20-PE-Vio615	for 100 tests	130-111-347
CD20-PE-Vio770	for 30 tests	130-111-526
CD20-PE-Vio770	for 100 tests	130-111-340
CD20-APC-Vio770	for 30 tests	130-111-527
CD20-APC-Vio770	for 100 tests	130-111-341
CD20-PerCP-Vio700	for 30 tests	130-111-528
CD20-PerCP-Vio700	for 100 tests	130-111-342
CD20-VioBright 515	for 30 tests	130-111-529
CD20-VioBright 515	for 100 tests	130-111-343
CD20-Biotin	for 30 tests	130-111-522

## 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

<b>Antigen</b>	CD20
<b>Clone</b>	REA780
<b>Isotype</b>	recombinant human IgG1

<b>Isotype control</b>	REA Control (S) antibodies
<b>Alternative names of antigen</b>	MS4A1, B1, Bp35, CVID5, LEU-16, MS4A2, S7, Ly-44
<b>Entrez Gene ID</b>	<a href="#">931</a>
<b>Molecular mass of antigen [kDa]</b>	33
<b>Cross-reactivity</b>	cynomolgus monkey ( <i>Macaca fascicularis</i> ), rhesus monkey ( <i>Macaca mulatta</i> )
<b>Distribution of antigen</b>	B cells, cancer stem cells, dendritic cells, lymphocytes
<b>Product format</b>	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Fixation</b>	The antibody is suited for staining of formaldehyde-fixed cells.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze.

Clone REA780 recognizes the CD20 antigen, a non-glycosylated transmembrane protein of 33–37 kDa that is expressed on B lineage cells from the pre-B cell stage to the B cell lymphoblast stage. The antigen is further expressed on most malignant B cells. CD20 is not found on early B cell progenitors or plasma cells. Oligomers of CD20 form a  $\text{Ca}^{2+}$  channel and might function in the regulation of local responses during B cell activation. Additional information: Clone REA780 displays negligible binding to Fc receptors.

## Reagent requirements

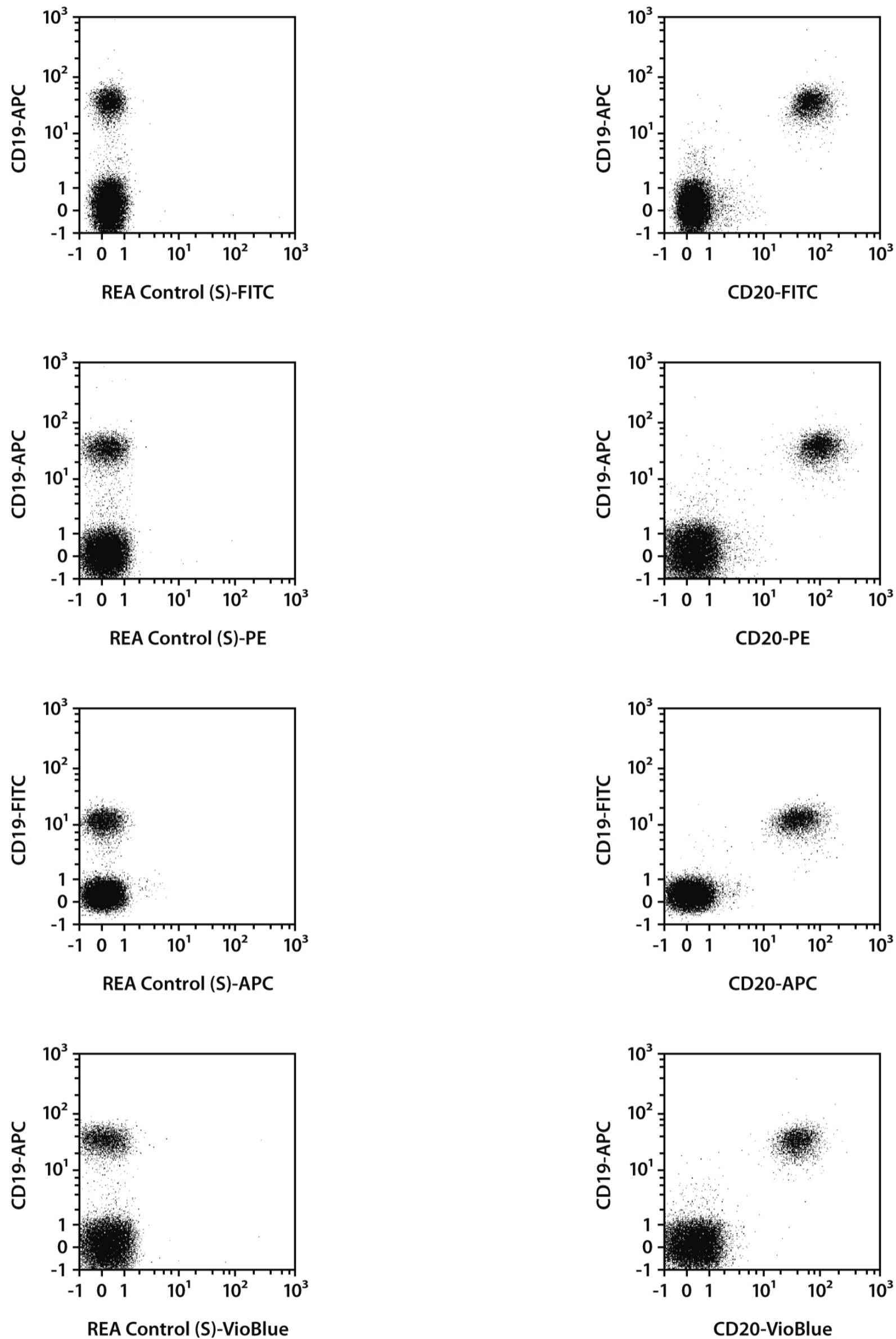
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  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.

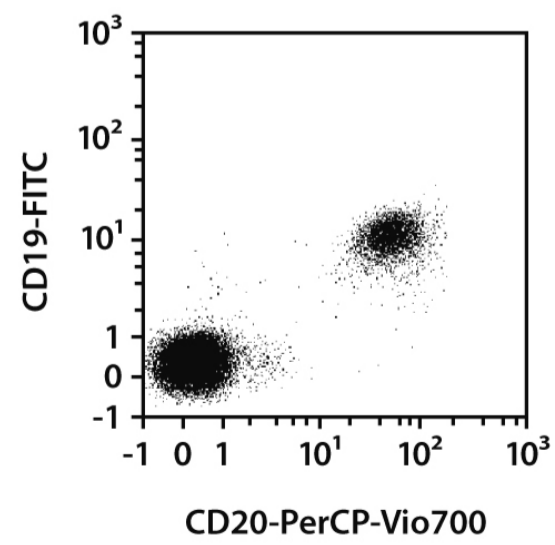
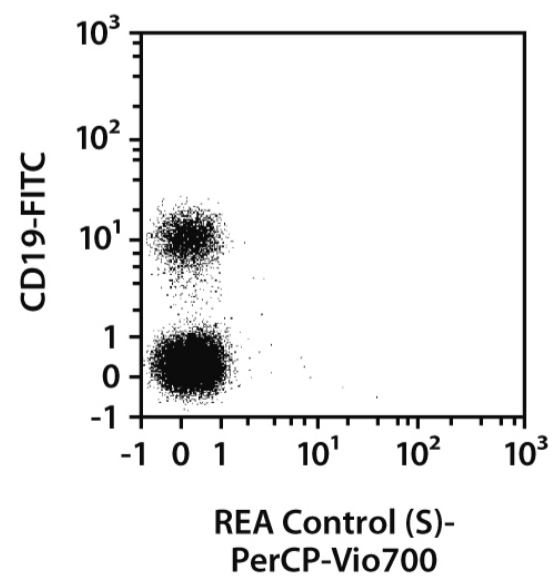
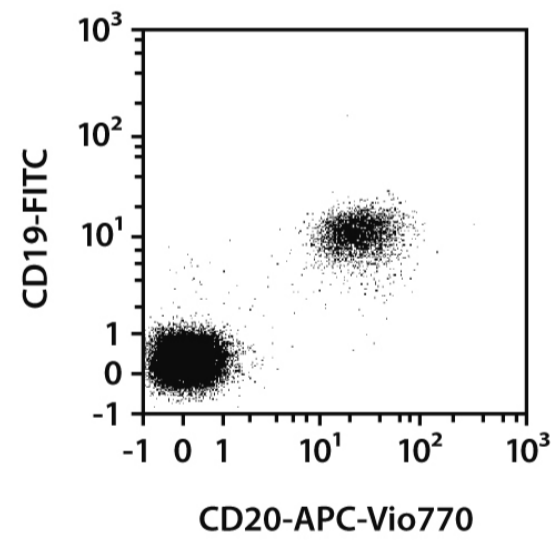
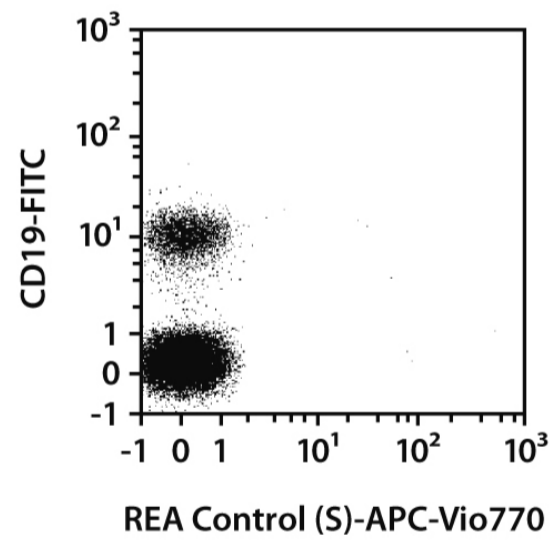
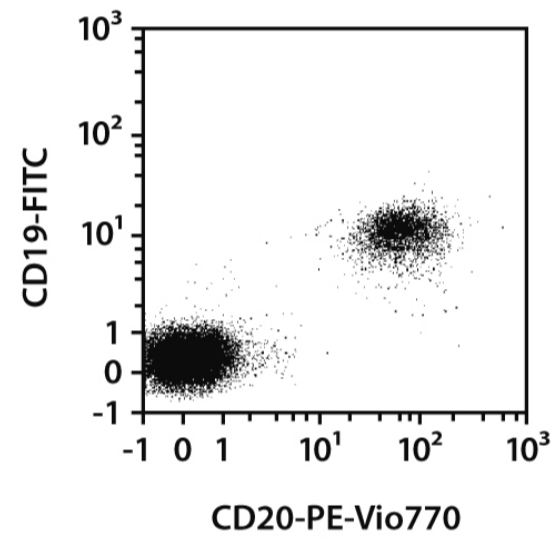
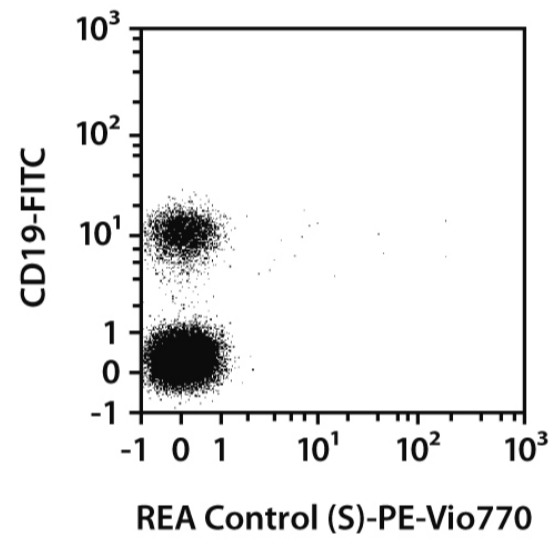
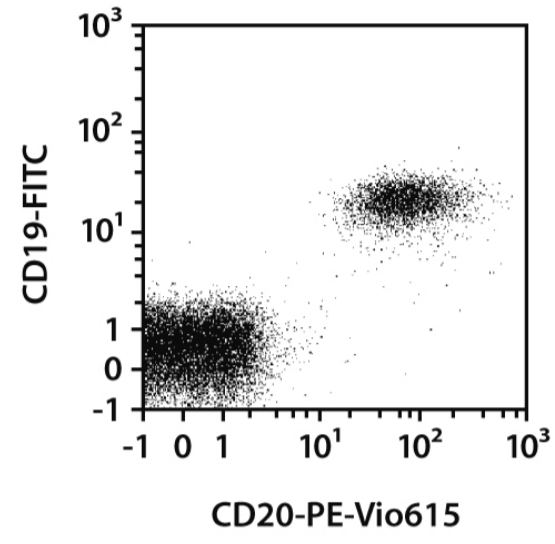
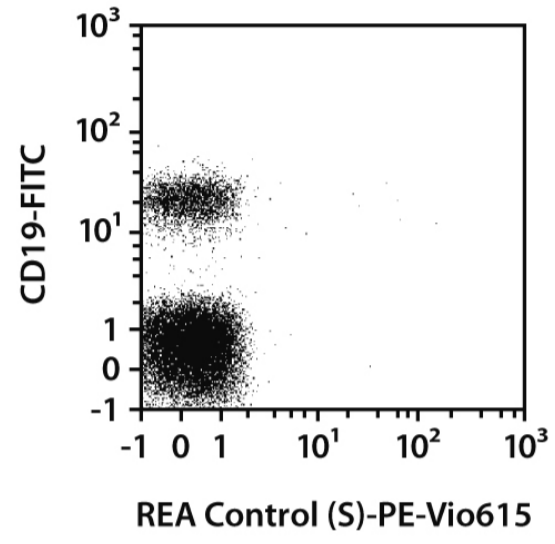
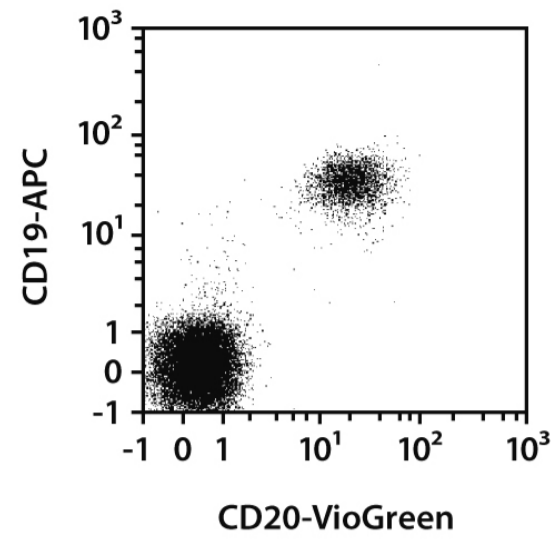
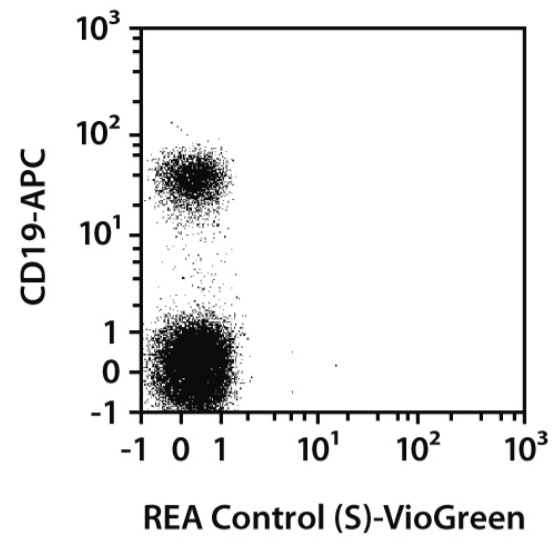
## Protocol for cell surface staining

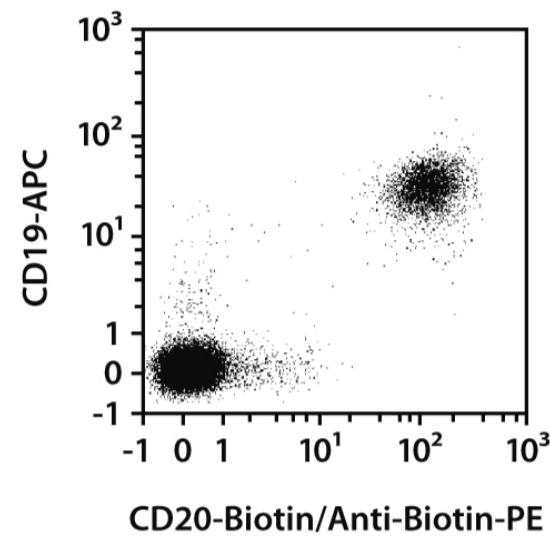
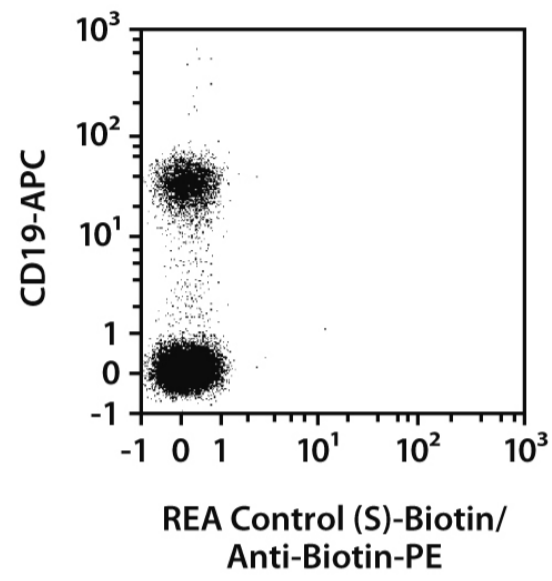
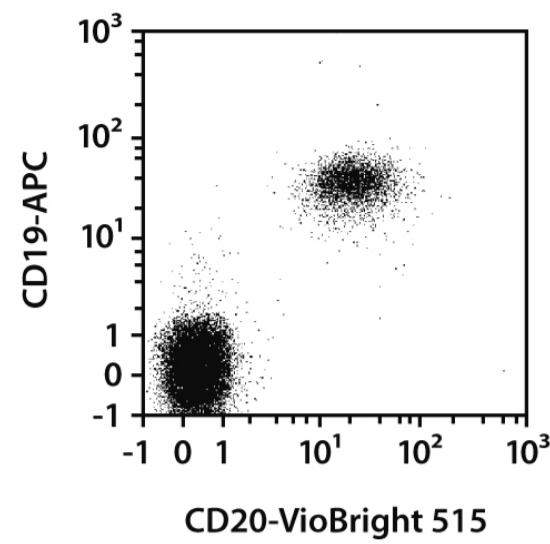
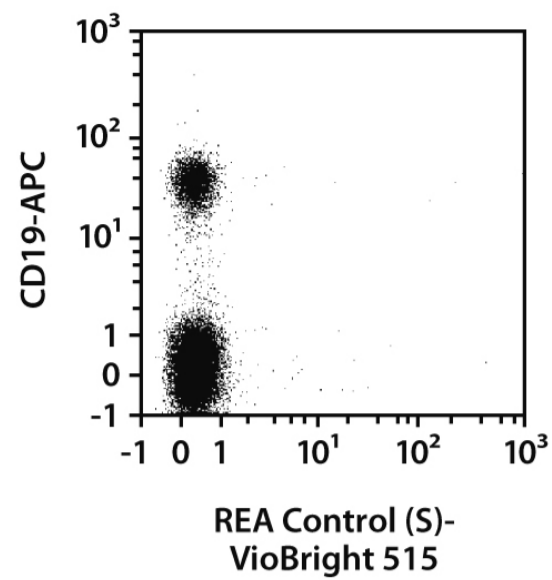
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to  $10^6$  cells/100  $\mu\text{L}$ .
  - Volumes given below are for up to  $10^6$  nucleated cells. When working with fewer than  $10^6$  cells, use the same volumes as indicated. When 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  $\mu\text{L}$  of buffer.
  4. Add 2  $\mu\text{L}$  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 CD20 antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD19 antibodies. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye-conjugated antibodies. Flow cytometry was performed 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.







## 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](mailto:macs@miltenyibiotec.de) | [www.miltenyibiotec.com](http://www.miltenyibiotec.com) Miltenyi Biotec provides products and services worldwide. Visit [www.miltenyibiotec.com/local](http://www.miltenyibiotec.com/local) to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. autoMACS, MACS, MACSQuant, Vio, VioBlue, VioBright, and VioGreen are either trademarks or registered trademarks of Miltenyi Biotec GmbH. Copyright © 2016 Miltenyi Biotec GmbH. All rights reserved.