



Miltenyi Biotec

CD31 antibodies, mouse

For research use only

30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD31-Biotin	150 µg in 1 mL	130-111-353
CD31-PE	30 µg in 200 µL	130-111-540
CD31-PE	150 µg in 1 mL	130-111-354
CD31-APC	30 µg in 200 µL	130-111-541
CD31-APC	150 µg in 1 mL	130-111-355
CD31-PE-Vio770	30 µg in 200 µL	130-111-542
CD31-PE-Vio770	150 µg in 1 mL	130-111-356
CD31-VioBright 515	30 µg in 200 µL	130-111-544
CD31-VioBright 515	150 µg in 1 mL	130-111-358
CD31-Biotin	30 µg in 200 µL	130-111-539

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	CD31
Clone	REA784
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	PECAM1, Pecam, EndoCAM, GPIIa
Entrez Gene ID	18613
Molecular mass of antigen [kDa]	79
Distribution of antigen	basophils, endothelial cells, fibroblasts, granulocytes, leukocytes, lymphocytes, macrophages, monocytes, neutrophils, NK cells, osteoblasts, plasma cells, platelets, T 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 REA784 recognizes the cell surface protein CD31. The murine CD31 antigen is also known as PECAM-1. The encoded protein is a single-pass type I membrane protein containing six Ig-like C2-type (immunoglobulin-like) domains and functions as cell adhesion molecule. CD31 is present on mature endothelial cells as well as on most leukocyte subtypes and platelets where

the expression level can vary. Besides its function in exhibiting adhesive properties, the protein is required for leukocyte transendothelial migration (TEM) under most inflammatory conditions. Additional information: Clone REA784 displays negligible binding to Fc receptors.

Reagent requirements

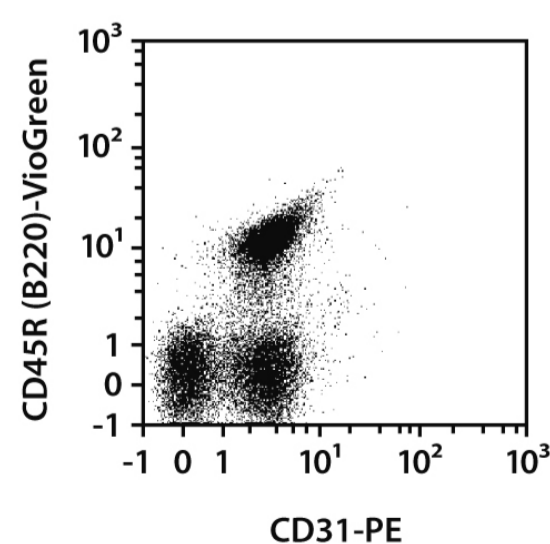
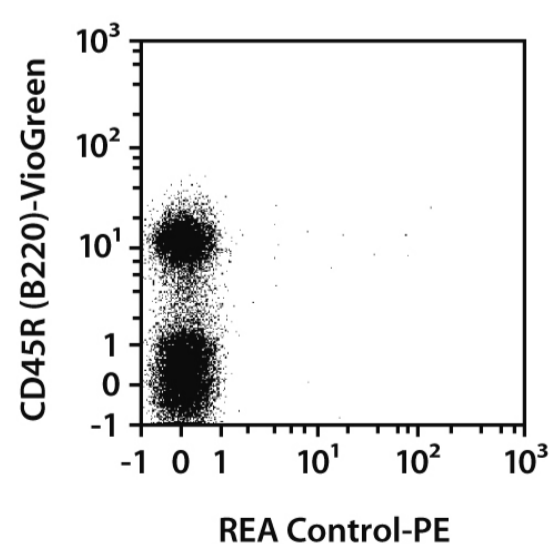
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ 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.

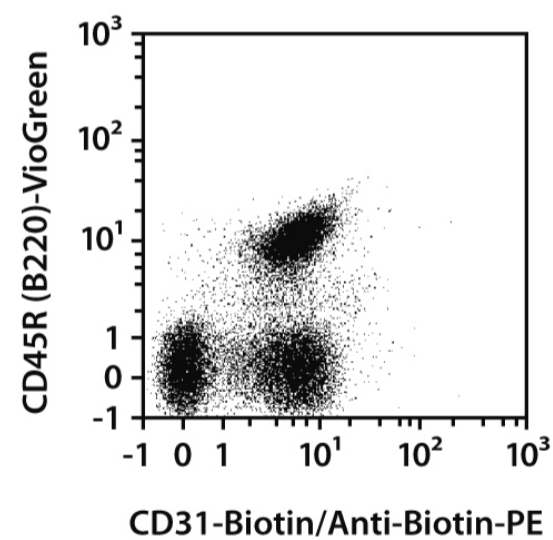
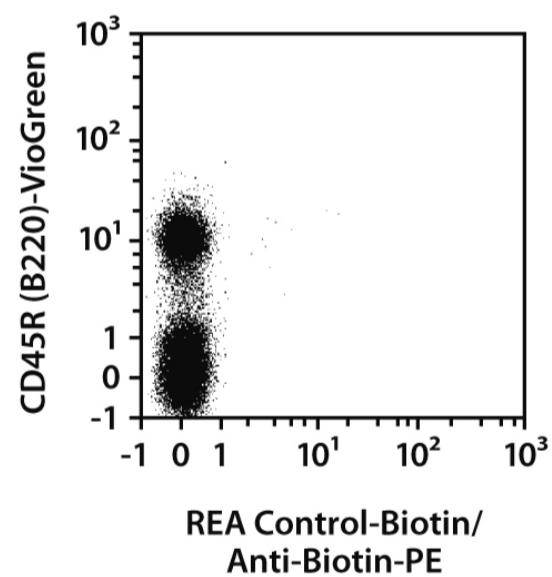
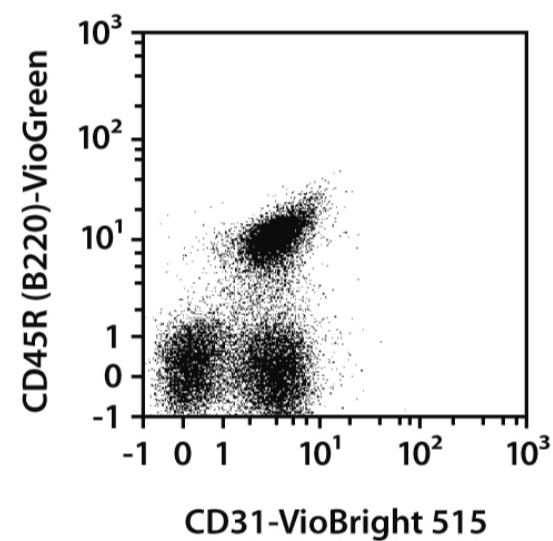
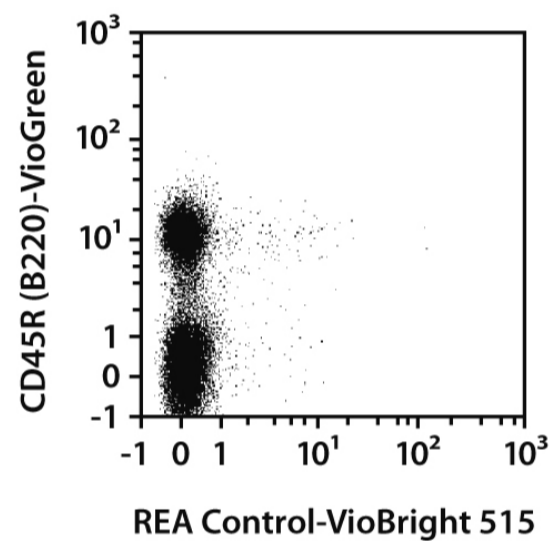
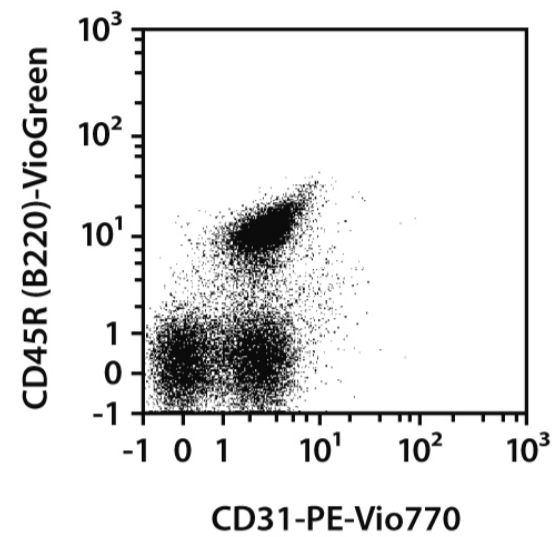
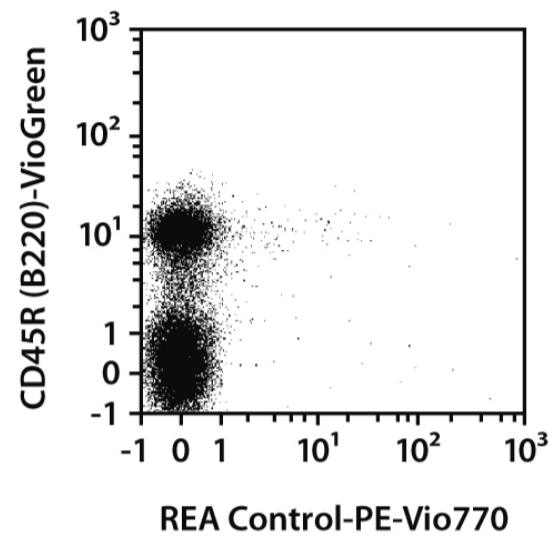
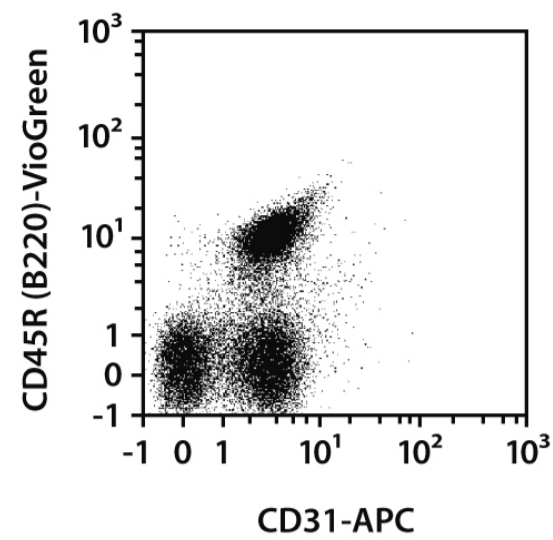
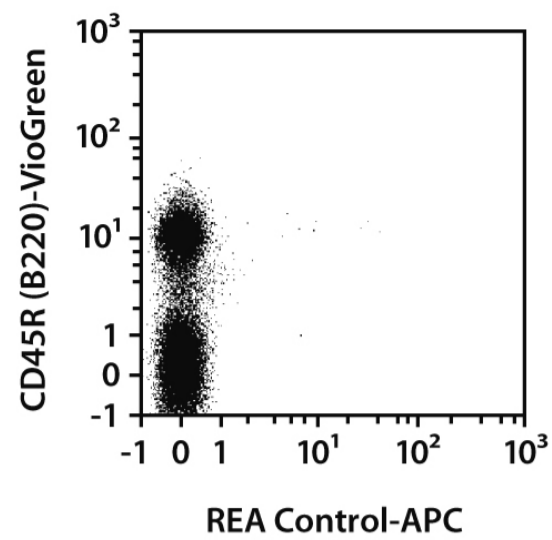
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10⁶ cells/100 µL.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ 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 10⁶ 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

Splenocytes from BALB/c mice were stained with CD31 antibodies or with the corresponding REA Control antibodies (left image) as well as with CD45R (B220) 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.





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