CD40 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.
CD40-APC-Vio770	for 30 tests	130-111-066
CD40-VioBright FITC	for 30 tests	130-111-067
CD40-VioBright FITC	for 100 tests	130-110-950
CD40-PE	for 30 tests	130-111-063
CD40-PE	for 100 tests	130-110-946
CD40-APC	for 30 tests	130-111-064
CD40-APC	for 100 tests	130-110-947
CD40-PE-Vio770	for 30 tests	130-111-065
CD40-PE-Vio770	for 100 tests	130-110-948
CD40-APC-Vio770	for 100 tests	130-110-949
CD40-Biotin	for 30 tests	130-111-062
CD40-Biotin	for 100 tests	130-110-945

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	CD40
Clone	REA733
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	Bp50, CD40L receptor, CDW40
Entrez Gene ID	958
Molecular mass of antigen [kDa]	28
Cross-reactivity	cynomolgus monkey (*Macaca fascicularis*), rhesus monkey (*Macaca mulatta*), baboon, chimpanzee (*Pan troglodytes*), squirrel monkey (*Saimiri sciureus*)
Distribution of antigen	B cells, macrophages, fibroblasts, smooth muscle
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 REA733 recognizes the human CD40 antigen, a transmembrane receptor of the tumor necrosis factor gene superfamily. CD40 is a co-stimulatory protein constitutively expressed by antigen presenting cells, including dendritic cells, B cells, and macrophages and on endothelial, smooth muscle cells, and fibroblasts. The binding of CD40 to CD154 (CD40L) on T helper cells activates antigen presenting cells and enhances the expression of cytokines, chemokines, matrix metalloproteinases, growth factors, and adhesion molecules. CD40 has been shown to interact with members of the TRAF family. Additional information: Clone REA733 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.

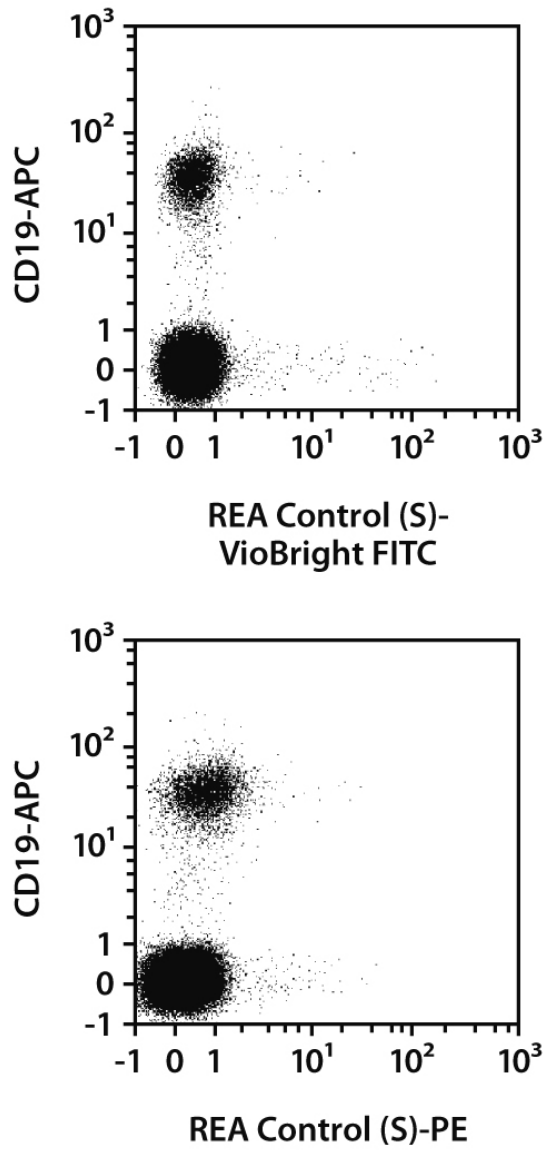
Protocol for cell surface staining

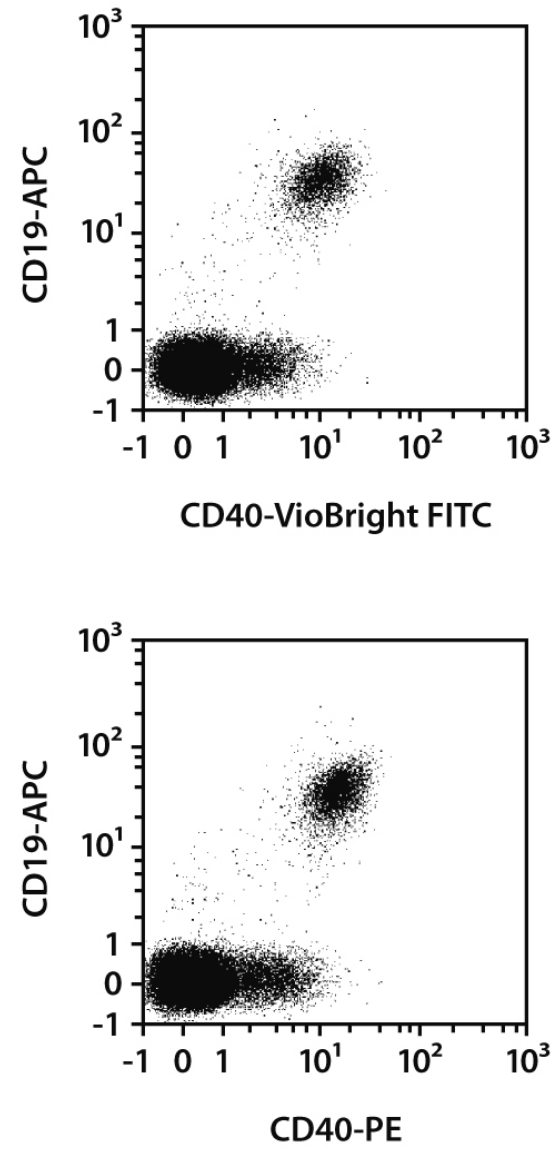
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

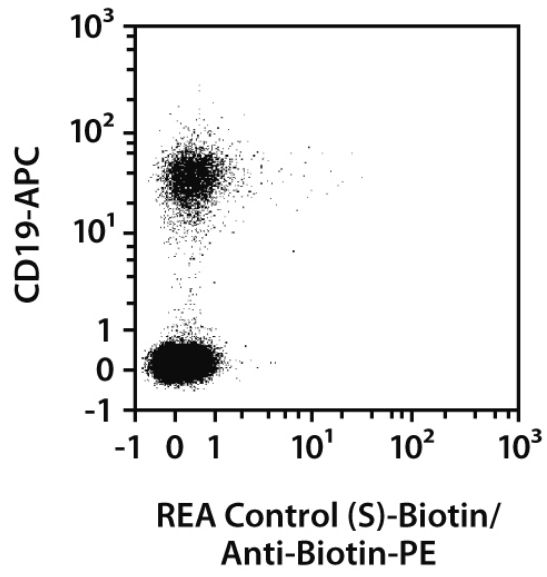
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

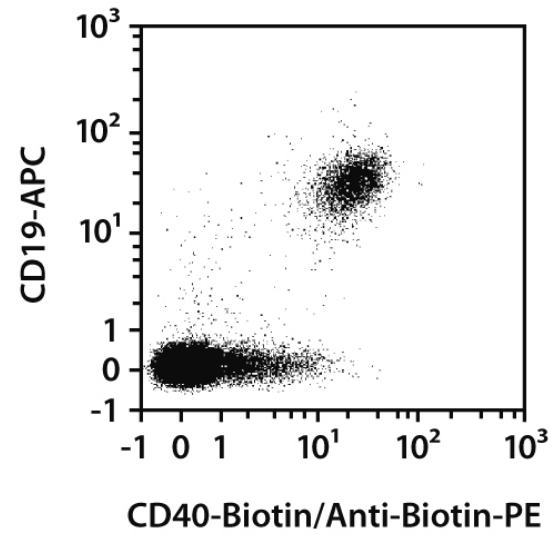
Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with CD40 antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD19 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.


CD19-APC
REA Control (S)-VioBright FITC
REA Control (S)-PE
CD19-FITC
REA Control (S)-APC
REA Control (S)-PE-Vio770
REA Control (S)-APC-Vio770


CD19-APC
CD40-VioBright FITC
CD40-PE
CD19-FITC
CD40-APC
CD40-PE-Vio770
CD40-APC-Vio770





Warranty

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