CD28 antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD28-VioBright FITC	for 30 tests	130-109-524
CD28-VioBright FITC	for 100 tests	130-109-445
CD28-PE	for 30 tests	130-109-520
CD28-PE	for 100 tests	130-109-441
CD28-APC	for 30 tests	130-109-521
CD28-APC	for 100 tests	130-109-442
CD28-PE-Vio770	for 30 tests	130-109-522
CD28-PE-Vio770	for 100 tests	130-109-443
CD28-APC-Vio770	for 30 tests	130-109-523
CD28-APC-Vio770	for 100 tests	130-109-444
CD28-Biotin	for 30 tests	130-109-519
CD28-Biotin	for 100 tests	130-109-440

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	CD28
Clone	REA612
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	T44, Tp44
Molecular mass of antigen [kDa]	23
Distribution of antigen	B cells, lymphocytes, plasma cells, T cells, thymocytes
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 REA612 recognizes the human CD28 antigen, a type I transmembrane protein, which is highly expressed on $CD3^+$ thymocytes, most peripheral thymocytes, and plasma cells but not in less mature B cells. Binding of CD28 to its ligands CD80 or CD86 costimulates T cell effector function and T

cell–dependent antibody production *in vitro* and *in vivo*. In the presence of antibodies directed against CD2 and CD3, CD28 antibodies stimulate T cell proliferation and cytokine production.
Additional information: Clone REA612 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.

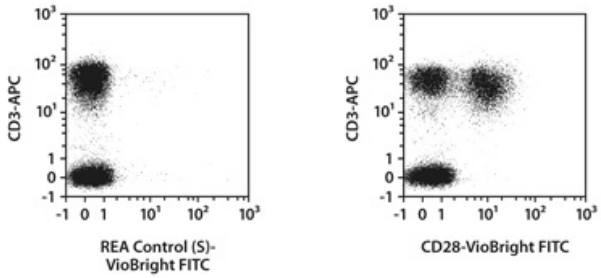
Protocol for cell surface staining

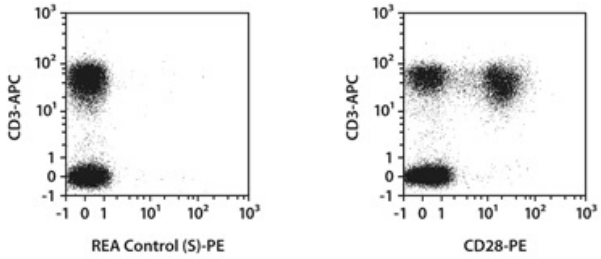
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

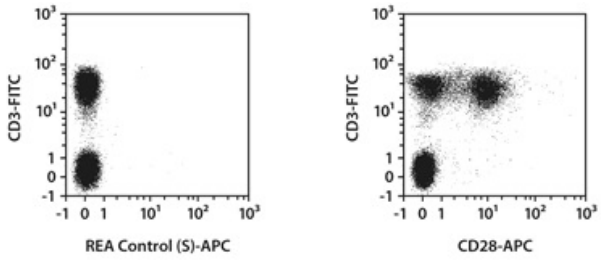
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

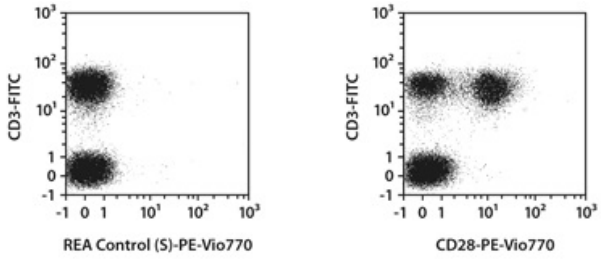
Examples of immunofluorescent staining

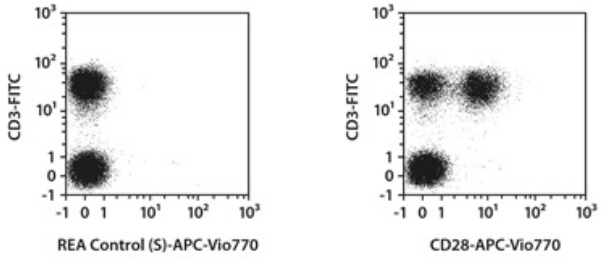
Human peripheral blood mononuclear cells (PBMCs) were stained with CD28 antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD3 antibodies. Flow cytometry was performed with the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding 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.

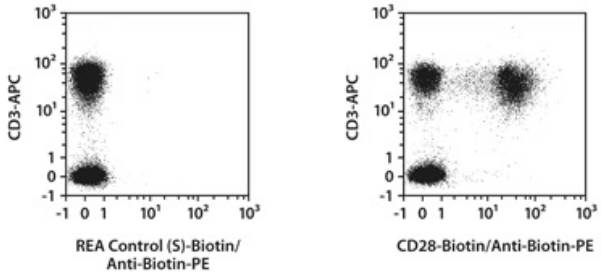

CD3-APC
REA Control (S)-
VioBright FITC
CD3-APC
CD28-VioBright FITC


CD3-APC
REA Control (S)-PE
CD3-APC
CD28-PE


CD3-FITC
REA Control (S)-APC
CD3-FITC
CD28-APC


CD3-FITC
REA Control (S)-PE-Vio770
CD3-FITC
CD28-PE-Vio770


CD3-FITC
REA Control (S)-APC-Vio770
CD3-FITC
CD28-APC-Vio770


CD3-APC
REA Control (S)-Biotin/
Anti-Biotin-PE
CD3-APC
CD28-Biotin/Anti-Biotin-PE

References

1. **Kozbor D. J. *et al.*** (1987) Tp44 molecules involved in antigen-independent T cell activation are expressed on human plasma cells. J. Immunol. 138(12): 4128–4132.
2. **June, C. H. *et al.*** (1990) Role of the CD28 receptor in T-cell activation. Immunol. Today 11(6): 211–216.
3. **Linsley, P. S. *et al.*** (1993) The role of the CD28 receptor during T cell responses to antigen. Annu. Rev. Immunol. 11: 191–212.
4. **Freeman, G. J. *et al.*** (1993) Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. Science 262(5135): 909–911.
5. **Krummel, M. F. and Allison, J. P.** (1995) CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J. Exp. Med. 182(2): 459–465.
6. **Bahlis, N. J. *et al.*** (2007) CD28-mediated regulation of multiple myeloma cell proliferation and survival. Blood 109(11): 5002–5010.

Warranty

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