CD28 antibodies, mouse

For research use only

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

Product	Content	Order no.
CD28-Biotin	30 µg in 200 µL	130-111-971
CD28-PE	30 µg in 200 µL	130-111-972
CD28-PE	150 µg in 1 mL	130-111-819
CD28-APC	30 µg in 200 µL	130-111-973
CD28-APC	150 µg in 1 mL	130-111-820
CD28-Biotin	150 µg in 1 mL	130-111-818

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	REA806
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	T44, Tp44
Entrez Gene ID	12487
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	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 REA806 recognizes the mouse CD28 antigen, a costimulatory molecule, expressed on most thymocytes, $CD4^+$ and $CD8^+$ T cells, and NK cells. Expression is down-regulated at late stages of terminal effector T cell differentiation. CD28 is a homodimeric type I transmembrane protein of the Ig receptor superfamily, composed of disulfide-linked 45 kDa subunits. Ligation of CD28 with CD80 (B7-1) and CD86 (B7-2) provides a costimulatory signal for T cell activation. Additional information: Clone REA806 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.

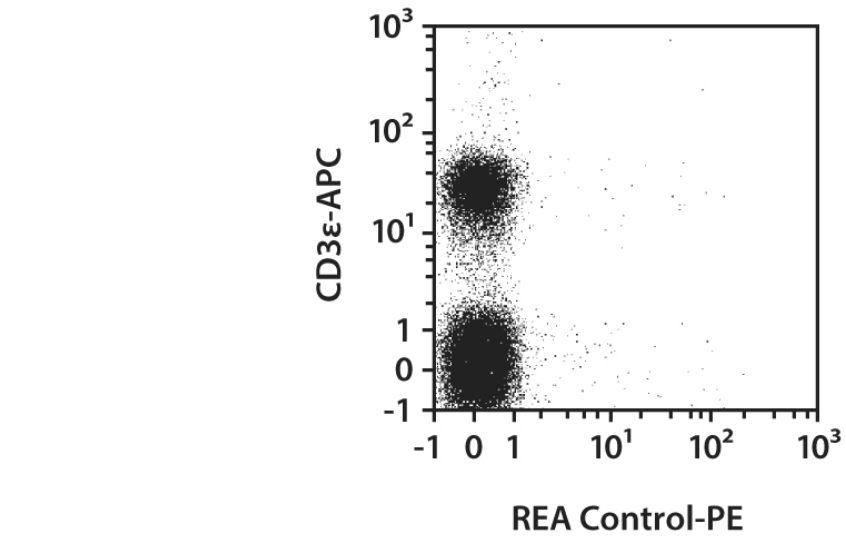
Protocol for cell surface staining

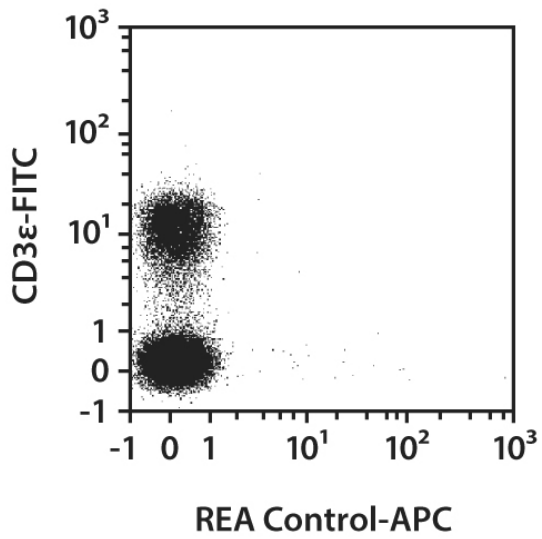
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

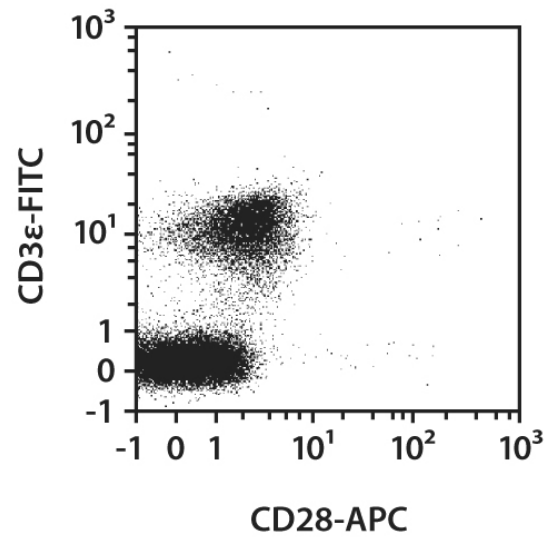
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

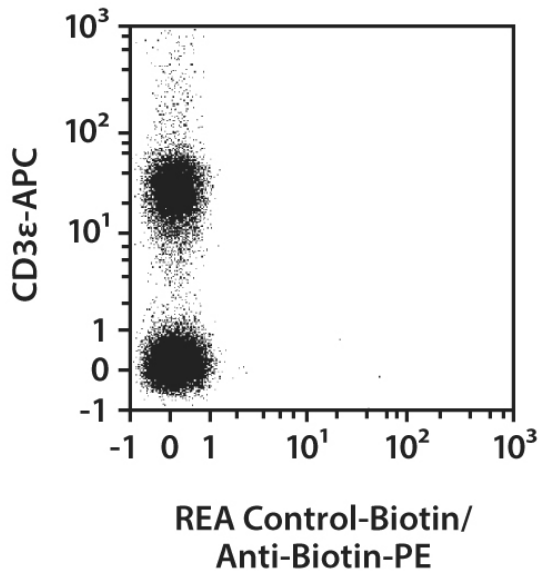
Examples of immunofluorescent staining

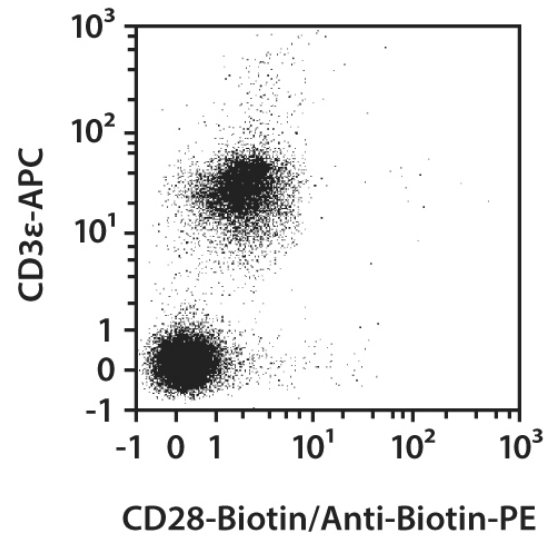
Splenocytes from BALB/c mice were stained with CD28 antibodies or with the corresponding REA Control antibodies (left image) as well as with CD3ε antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.











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