CD69 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.
CD69-FITC	30 µg in 200 µL	130-115-574
CD69-FITC	150 µg in 1 mL	130-115-459
CD69-PE	30 µg in 200 µL	130-115-575
CD69-PE	150 µg in 1 mL	130-115-460
CD69-APC	30 µg in 200 µL	130-115-576
CD69-APC	150 µg in 1 mL	130-115-461
CD69-PE-Vio770	30 µg in 200 µL	130-115-577
CD69-APC-Vio770	30 µg in 200 µL	130-115-578

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	CD69
Clone	REA937
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	AIM, VEA, EA1, MLR3, gp34/28
Entrez Gene ID	12515
Molecular mass of antigen [kDa]	22
Distribution of antigen	B cells, granulocytes, NK cells, 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 REA937 recognizes the mouse CD69 antigen, a type II integral membrane protein with a C-type lectin domain. CD69 is expressed as a homodimer composed of heavily glycosylated subunits. It is rapidly induced upon activation of T and B cells, neutrophils, and NK cells, which is why CD69 has been mostly regarded as an activation marker. The precise role of CD69 in immunity has not been determined because its ligand is unknown. Freshly prepared thymocytes undergoing selection events express CD69, and regulatory roles for CD69 expression in T cell development in the thymus have been suggested. However, phenotypical analysis in previous studies using CD69-deficient mice has revealed that CD69 does not appear to be required for the development of CD4 T cells. CD69 is also expressed on platelets. Recent studies have shown that CD69 is constitutively expressed by tissue-resident T_H memory cells and that its function is essential for the generation of professional resting

memory TH cells. Additional information: Clone REA937 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.

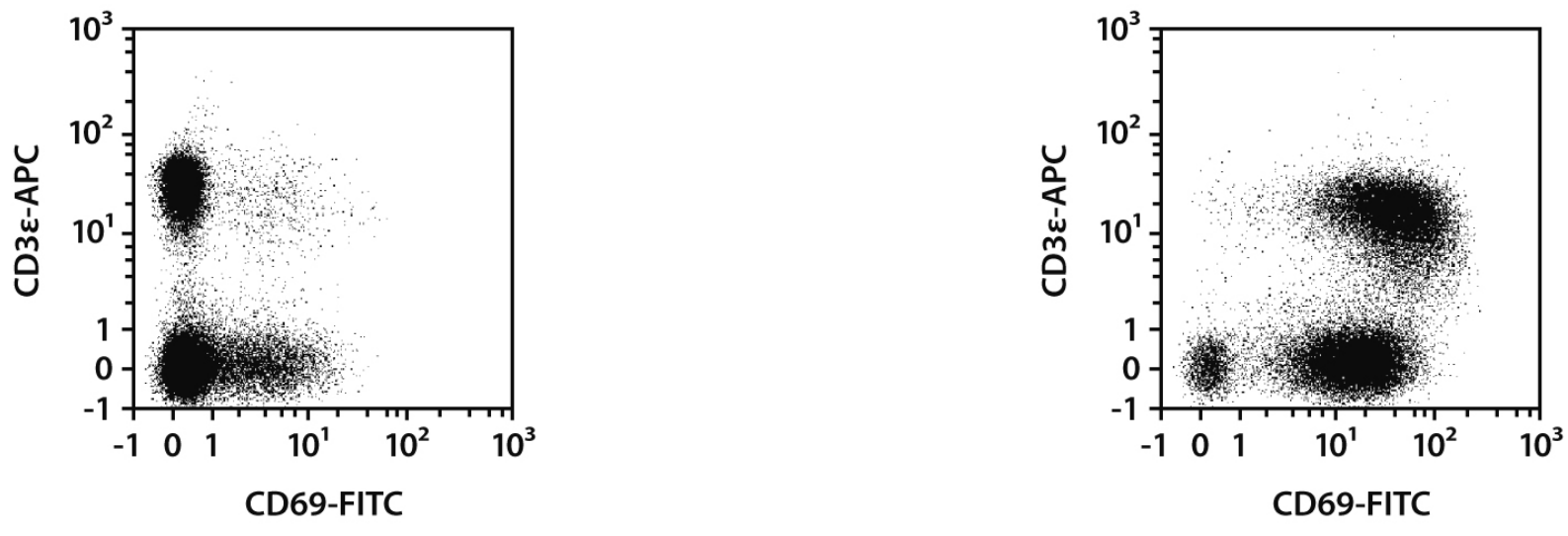
Protocol for cell surface staining

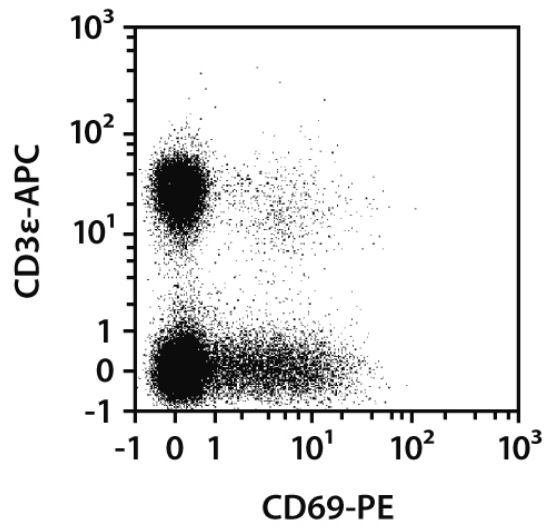
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell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

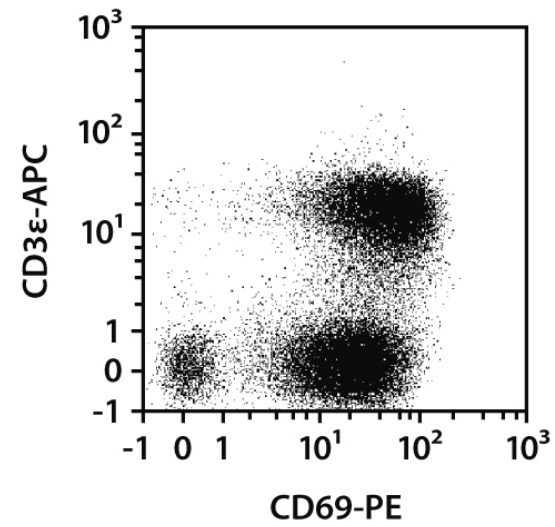
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher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

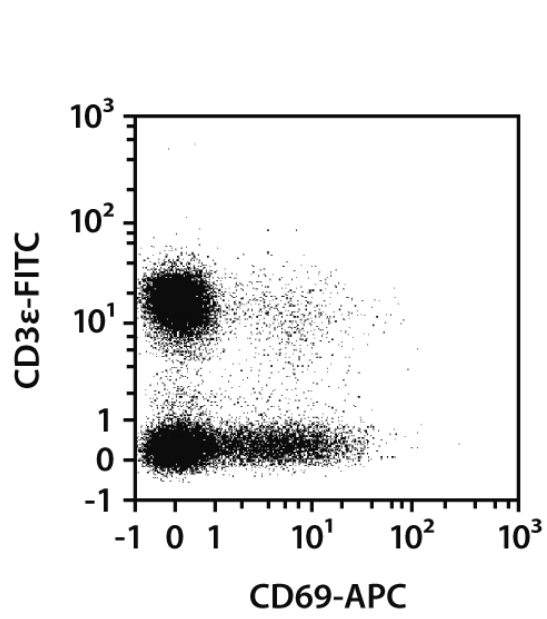
Examples of immunofluorescent staining

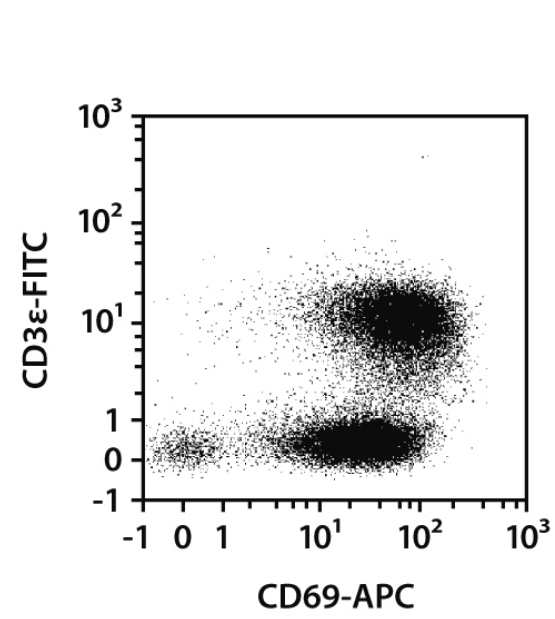
Splenocytes from BALB/c mice, either unstimulated (left images) or stimulated with 10 ng/mL PMA for 16 hours, were stained with CD69 antibodies as well as with CD3ε antibodies. Flow cytometry was performed usign the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

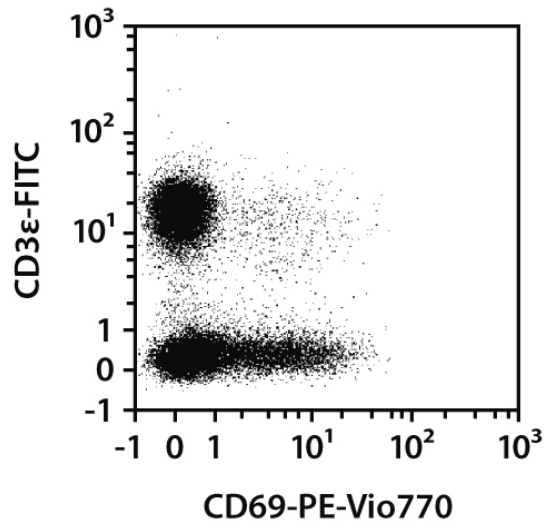


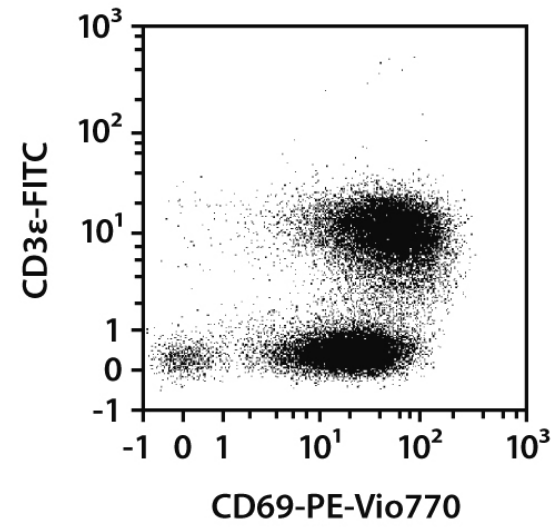


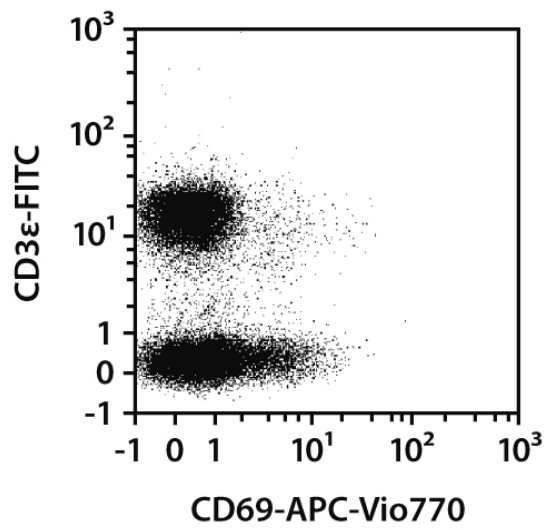


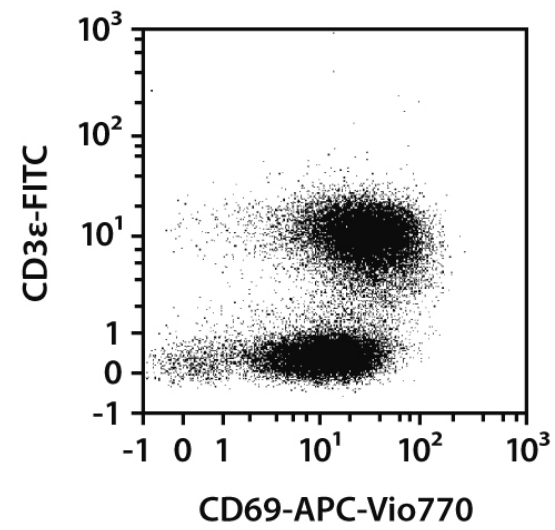












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