CD69 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD69-FITC	9 µg in 300 µL	130-103-983
CD69-FITC	30 µg in 1 mL	130-103-950
CD69-PE	9 µg in 300 µL	130-103-979
CD69-PE	30 µg in 1 mL	130-103-946
CD69-APC	9 µg in 300 µL	130-103-980
CD69-APC	30 µg in 1 mL	130-103-947
CD69-VioBlue	9 µg in 300 µL	130-103-982
CD69-VioBlue	30 µg in 1 mL	130-103-949
CD69-VioGreen	9 µg in 300 µL	130-103-985
CD69-VioGreen	30 µg in 1 mL	130-103-952
CD69-PE-Vio770	9 µg in 300 µL	130-103-977
CD69-PE-Vio770	30 µg in 1 mL	130-103-944
CD69-APC-Vio770	9 µg in 300 µL	130-103-984
CD69-APC-Vio770	30 µg in 1 mL	130-103-951
CD69-PerCP-Vio700	9 µg in 300 µL	130-103-978
CD69-PerCP-Vio700	30 µg in 1 mL	130-103-945
CD69-Biotin	9 µg in 300 µL	130-103-981
CD69-Biotin	30 µg in 1 mL	130-103-948

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	H1.2F3
Isotype	hamster IgG1
Alternative names of antigen	AIM, VEA, EA1, MLR3, gp34/28
Molecular mass of antigen [kDa]	22
Distribution of antigen	T cells, B cells, NK cells, neutrophils
Product format	Antibodies 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 H1-2F3 recognizes mouse CD69, a type II integral membrane protein with a C-type lectin domain. CD69 is expressed as a homodimer composed of heavily glycosylated subunits. CD69 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 Th memory cells and that its function is essential for the generation of professional resting memory Th cells.

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) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (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 1:10 for up to 10^6 cells/50 µL of buffer.
- 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 (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

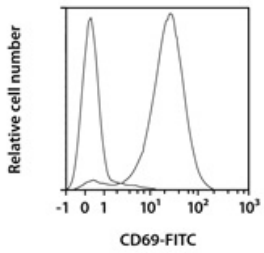
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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

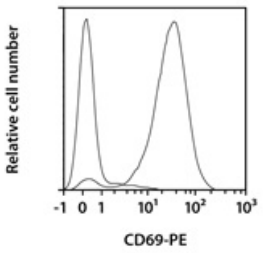
Splenocytes from BALB/c mice, either left unstimulated (left peak) or stimulated with 10 ng/mL Phorbol 12-myristate 13-acetate (PMA) for 15 hours, were stained with CD69 antibodies conjugated to FITC (A),

PE (B), APC (C), VioBlue (D), VioGreen (E), PE-Vio770 (F), APC-Vio770 (G), PerCP-Vio700 (H) and Biotin (I), and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

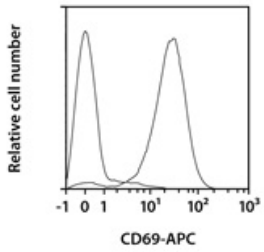
A:



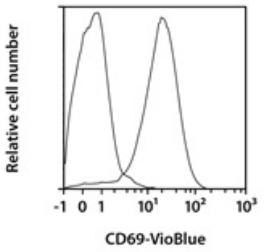
B:



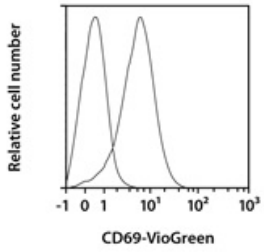
C:



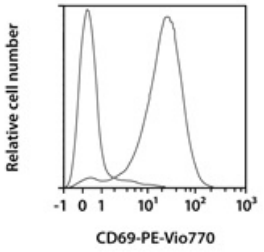
D:



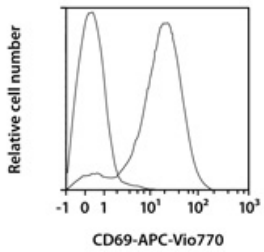
E:



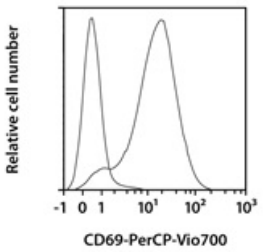
F:



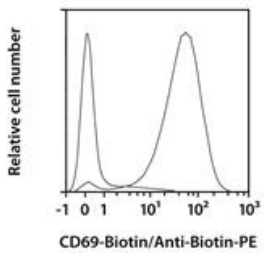
G:



H:



I:



References

1. **Sathaliyawala, T. *et al.*** (2013) Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. Immunity 38(1): 187–197.
2. **Shinoda, K. *et al.*** (2012) Type II membrane protein CD69 regulates the formation of resting T-helper memory. Proc. Natl. Acad. Sci. U.S.A. 109(19): 7409–7414.
3. **Sancho, D. *et al.*** (2005) CD69 is an immunoregulatory molecule induced following activation. Trends Immunol. 26(3): 136–140.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | **www.miltenyibiotec.com**
Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.