Miltenyi Biotec

CD69 antibodies
human

Contents

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.

1. Description

This product is for research use only.

Components Monoclonal CD69 antibodies, human:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-092-166	130-098-901
PE	130-092-160	130-099-382
APC	130-092-159	130-098-893
PE-Vio® 615	130-106-552	130-106-596
PE-Vio® 770	130-099-749	130-099-750
APC-Vio® 770	130-099-907	130-099-904
Biotin	130-092-161	130-099-111

Clone FN50 (isotype: mouse IgG1).

Capacity 1 mL: 100 tests or up to 10^9 total cells.

300 µL: 30 tests or up to 3×10^8 total cells.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

Cross-reactivity: The CD69 antibody has been reported to react with

- rhesus monkey (*Macaca mulatta*) cells
- cynomolgus monkey (*Macaca fascicularis*) cells
- peripheral blood lymphocytes (PBLs) from baboon[1]

1.1 Background information

- Antigen: CD69
- Synonym: activation inducer molecule (AIM)
- Expression patterns: CD69 is involved in the early events of lymphocyte, monocyte, and platelet activation. Cross-linking of CD69 induces cytotoxic activity and costimulates cytokine production of activated NK cells and selected T cell clones. CD69 is transiently expressed on activated leukocytes including T cells, thymocytes, B cells, NK cells, neutrophils, and eosinophils. It is constitutively expressed by a subset of medullary mature thymocytes, platelets, mantle B cells, and certain $CD4^+$ T cells in germinal centers of normal lymph nodes.

1.2 Applications

- Identification and enumeration of CD69-producing cells.
- Studies of T cell activation and analysis of cytokine expression in combination with cytokine secretion assays or intracellular cytokine staining.

1.3 Recommended antibody dilution

- CD69 antibodies should be used at a dilution of 1:11.
- The FITC-conjugated antibody is suited for staining of formaldehyde-fixed cells.

1.4 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.

- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells containing Inside Fix and Inside Perm.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated antibodies for cell surface staining or for intracellular staining of activation markers. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

140-001-502.05

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- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD69-Biotin.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. Protocols

▲ It is recommended to stain 10^6 cells per sample. When working with up to 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).

2.1 Cell surface staining

1. Wash up to 10^7 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend up to 10^7 cells per 100 µL of buffer.
3. Add 10 µL of the CD69 antibody.
4. 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.
5. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. (Optional) If Anti-CD69-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody, and continue as described in steps 4 and 5.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

2.2 Intracellular staining of cells in suspension

1. Wash up to 10^7 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 cells in 500 µL of buffer.
4. Add 500 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes in the dark at room temperature.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.

   ▲ **Note:** Fixed cells may be stored in azide-containing buffer at 2–8 °C for up to 1 week.
7. (Optional) Stain cell surface antigens that are sensitive to permeabilization with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cells in 90 µL of Inside Perm. Add 10 µL of the CD69 antibody.

   ▲ **Note:** For staining with several antibodies in this step, reduce the volume of Inside Perm accordingly. For efficient permeabilization, the volume of Inside Perm should be at least 30% of the overall staining volume.
10. Mix well and incubate for 10 minutes in the dark at room temperature.
11. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
12. (Optional) If CD69-Biotin was used, resuspend cell pellet in 100 µL of Inside Perm, add 10 µL of anti-biotin antibody, and continue as described in steps 9 and 10.
13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

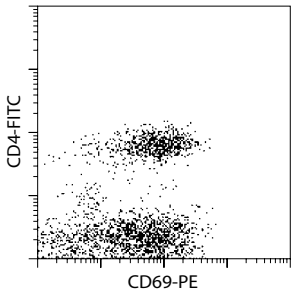
    ▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.

    ▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

3. Examples of immunofluorescent staining with CD69 antibodies

A) Cell surface staining with CD69 antibodies

Peripheral blood mononuclear leukocytes were stimulated *in vitro* with Cytostim (# 130-092-172) for 2 hours. The cells were harvested, stained with CD69-PE as well as with CD4-FITC (# 130-080-501), and analyzed by flow cytometry. A lymphocyte gate based on forward and side scatter properties was activated. Cell debris and dead cells were excluded from the analysis based on propidium iodide fluorescence.



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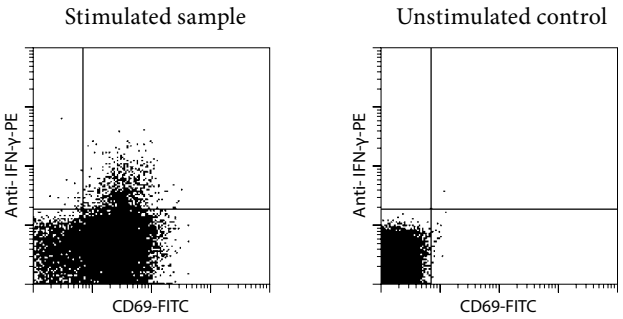
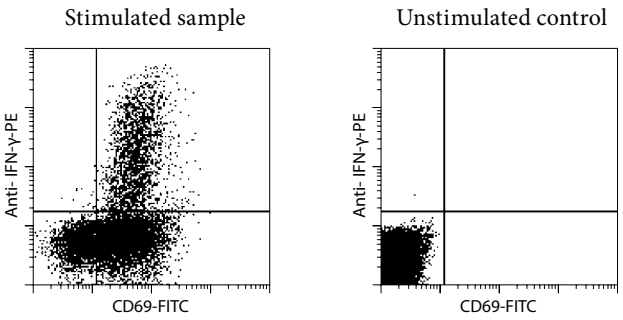
B) Cell surface staining with CD69 antibodies in combination with the IFN-γ Secretion Assay–Detection Kit (PE)

Peripheral blood mononuclear leukocytes were stimulated *in vitro* with CytoStim (# 130-092-172) for 2 hours. The cells were harvested and the IFN-γ Secretion Assay–Detection Kit (PE) (# 130-054-202) protocol was performed. Subsequently, the cells were counterstained with CD69-FITC and CD8-APC (# 130-091-076) and analyzed by flow cytometry. Dot plots show staining of CD69 versus IFN-γ on viable $CD8^+$ lymphocytes gated according to scatter properties and CD8 and propidium iodide fluorescence.



C) Intracellular staining with CD69 antibodies in combination with Anti-IFN-γ-PE

PBMCs were stimulated *in vitro* with CytoStim (# 130-092-172) for 6 hours. Brefeldin A was added after 2 hours. The cells were harvested, fixed, stained with CD8-APC, permeabilized, and intracellularly stained with CD69-FITC and Anti-IFN-γ-PE. Cells were analyzed by flow cytometry. Analysis was restricted to viable CD8 lymphocytes based on CD8 expression and side scatter properties. Cell debris and dead cells were excluded from the analysis based on propidium iodide fluorescence.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. Reference

1. Lin, G. X. *et al.* (2002) Cross-reactivity of CD antibodies in eight animal species. In: Mason (editor): Leucocyte Typing VII. Oxford, Oxford University Press; pp. 519–524.

Refer to www.miltenyibiotec.com for all data sheets and protocols.