Anti-Perforin antibodies
human

Contents

1. Description

Components 1 mL monoclonal Anti-Perforin antibodies, human conjugated to various dyes.

VioBlue®	130-096-671
FITC	130-096-668
PE	130-096-578
APC	130-096-569
Biotin	130-096-760

Clone delta G9 (isotype: mouse IgG2b).

Capacity 100 tests or up to 10^9 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.

1.1 Background information

The clone delta G9 reacts with human perforin, a 70 kD cytolytic protein, which is expressed by cytotoxic T lymphocytes and natural killer (NK) cells, and which is stored in cytoplasmic granules. Upon cell activation perforin is released and functions as one of the major effector molecule to mediate killing of target cells.

1.2 Applications

- Identification and enumeration of perforin-producing cells including T cells.

1.3 Recommended antibody dilution

- Anti-Perforin antibodies should be used at a dilution of 1:10.

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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-Perforin-Biotin.
- (Optional) Fluorochrome-conjugated antibodies for cell surface staining, e.g., CD8-APC (# 130-091-076) or CD8-FITC (# 130-080-601). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Additional requirements for intracellular staining in combination with magnetic cell separation (refer to protocol 2.2.2)

- MACS MicroBeads of choice.
- MS Columns and suitable MACS Separator (MiniMACS™, OctoMACS™, VarioMACS™, or SuperMACS™ II Separator).

  ▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS or SuperMACS II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

140-003-357.01

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2. Protocols

2.1 Sample preparation

To detect perforin-positive cells, best results are achieved by starting the assay with fresh PBMCs or with other leukocyte-containing single-cell preparations from tissues or cell lines. Alternatively, frozen cell preparations can be used. For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

2.2 Intracellular immunofluorescent staining protocols

2.2.1 Intracellular staining of cells in suspension

▲ 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).

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 Anti-Perforin antibody.
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 Anti-Perforin-Biotin was used, resuspend cell pellet in 100 µL of Inside Perm, add 10 µL of anti-biotin antibody, and continue as described in step 10 and 11.
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.

2.2.2 Intracellular staining in combination with magnetic cell separation (solid phase intracellular staining)

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
3. Add 20 µL of MACS MicroBeads, e.g., CD8 MicroBeads (# 130-045-201), per 10^7 total cells.

   ▲ **Note:** For details on the procedure refer to the respective MACS MicroBeads data sheet.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) Counterstain cell surface antigens with antibodies that are sensitive to fixation according to the manufacturer's recommendations.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

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7. Resuspend cells in 500 µL of buffer.
8. Place MS Column in the magnetic field of a suitable MACS Separator.
9. Prepare column by rinsing with 500 µL of buffer.
10. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
11. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with effluent from step 10.
12. Remove column from the separator and place it on a suitable collection tube.
13. Pipette 500 µL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
14. Add 500 µL of Inside Fix to the eluted cell fraction and incubate for 20 minutes at room temperature.
15. Place a second MS Column in the magnetic field of a suitable MACS Separator and prepare column by rinsing with 500 µL of buffer.
16. Apply the fixed cell suspension onto the column.
17. Wash cells by rinsing the column with 1×500 µL of buffer, followed by 2×500 µL of Inside Perm.
18. Prepare a solution of 10 µL of Anti-Perforin antibodies and 90 µL of Inside Perm.
19. (Optional) Add additional staining antibodies to the solution, e.g., for the staining of cell surface antigens internalized upon cell activation or antigens which accumulate in the cell.

    ▲ **Note:** Do not exceed the total solution volume of 150 µL.
20. Apply the solution onto the column and incubate for 10 minutes at room temperature.

    ▲ **Note:** The MACS Column has a flow-stop mechanism that will retain the solution in the column.
21. Wash cells by rinsing the column with 2×500 µL of Inside Perm followed by 1×500 µL of buffer.
22. Remove column from the separator and place it on a suitable collection tube.
23. Pipette 500 µL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
24. Cells are now ready for analysis. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

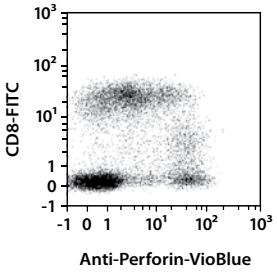
    ▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.

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

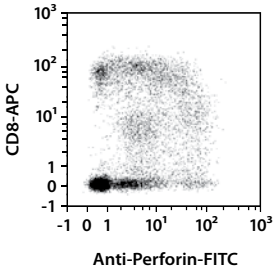
3. Examples of immunofluorescent staining with Anti-Perforin antibodies

Fixed and permeabilized human peripheral blood mononuclear cells (PBMCs) were stained with Anti-Perforin antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) as well as with CD8-APC (#130-091-076) or CD8-FITC (#130-080-601) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-Perforin-Biotin (E) were stained with Anti-Biotin-PE (#130-090-756). Gating was performed on lymphocytes according to scatter properties. Cell debris was excluded from the analysis based on scatter signals and in a fluorescence 3 versus fluorescence 4 dot plot.

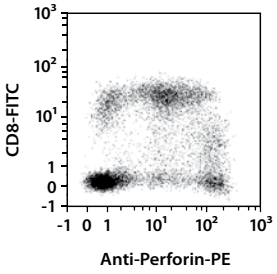
A)



B)



C)



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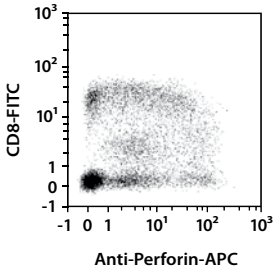
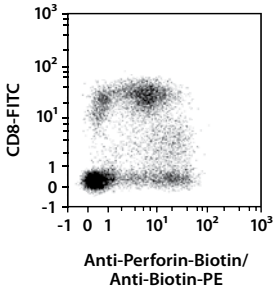
D)



E)



All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

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