Miltenyi Biotec

Blood Dendritic Cell Phenotyping Cocktail Kit, anti-human

for 50 tests 130-091-086
for 2×50 tests 130-091-324

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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

1 mL BDC Phenotyping Cocktail, anti-human containing:

CD1c (BDCA-1) Antibody, anti-human, PE (clone: AD5-8E7, isotype: mouse IgG2a)

CD303 (BDCA-2) Antibody, anti-human, FITC (clone: AC144, isotype: mouse IgG1)

CD141 (BDCA-3) Antibody, anti-human, APC (clone: AD5-14H12, isotype: mouse IgG1)

CD20 Antibody, anti-human, PerCP (clone: LT20, isotype: mouse IgG1κ)

CD14 Antibody, anti-human, PerCP (clone: TÜK4, isotype: mouse IgG2aκ)

1 mL BDC Control Cocktail, anti-human containing:

Isotype Control Antibody, mouse IgG2a, PE (clone: S43.10, isotype: mouse IgG2a)

Cytokeratin Antibody, anti-human, FITC (clone: CK3-6H5, isotype: mouse IgG1κ)

Biotin Antibody, APC (clone: Bio3-18E7, isotype: mouse IgG1)

CD20 Antibody, anti-human, PerCP (clone: LT20, isotype: mouse IgG1κ)

CD14 Antibody, anti-human, PerCP (clone: TÜK4, isotype: mouse IgG2aκ)

50 mL Red Blood Cell Lysis Solution (10×)

1 mL Dead Cell Discriminator

0.5 mL Discriminator Stop Reagent

25 mL Fix Solution

Capacity 50 tests or 2×50 tests. One test corresponds to fluorescent labeling of 2×300 µL whole anticoagulated blood or 2×10^6 PBMCs.

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 Blood Dendritic Cell Phenotyping Cocktail Kit, anti-human is designed for easy identification and enumeration of dendritic cells (DCs) and DC subsets in whole blood or PBMCs by flow cytometry. The kit is based on the DC-specific surface antigens CD303 (BDCA-2), CD141 (BDCA-3), and CD1c (BDCA-1). The markers allow easy identification of three distinct DC subsets in blood: Plasmacytoid DCs (PDCs) are identified by CD303 (BDCA-2), type-1 myeloid DCs (MDC1s) are identified by CD1c (BDCA-1), and type-2 myeloid DCs (MDC2s) are identified by CD141 (BDCA-3) expression. The dead cell dye used in this kit allows photolabeling of dead cells in combination with cell fixation. This is particularly useful when working with hazardous samples.

1.2 Applications

- Identification and enumeration of DCs and DC subsets in whole blood or peripheral blood mononuclear cells (PBMCs).

1.3 Reagent and instrument 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.

- Flow cytometer equipped with a red (640 nm) and a blue (488 nm) laser, e.g., MACSQuant® Analyzer 16 (# 130-109-803).
- FcR Blocking Reagent, human (# 130-059-901) for analysis of PBMCs.
- Double distilled water (ddH_2O).
- Light source for photolabeling of dead cells using the Dead Cell Discriminator.

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- (Optional) MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

2.1 Immunofluorescent staining of nucleated cells, e.g., PBMCs

▲ Volumes given below are for **up to 10^6** nucleated 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Label two tubes with A (sample) or B (control).
2. Carefully add 10^6 PBMCs in 240 µL of buffer to the bottom of each tube.
3. Add 60 µL of FcR Blocking Reagent, human to each tube.
4. Add 20 µL BDC Phenotyping Cocktail, anti-human to tube A and 20 µL BDC Control Cocktail, anti-human to tube B.
5. Add 10 µL Dead Cell Discriminator to each tube.
6. Mix gently and incubate for 10 minutes in a horizontal position on ice under a light source (distance 3–5 cm).

   ▲ **Note:** Dead Cell Discriminator is a fluorescent photolytic dye that needs exposure to light for irreversible binding. Refer to the data sheet of the Fixation and Dead Cell Discrimination Kit (# 130-091-163) for more information.
7. Add 4 mL of buffer.
8. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
9. Resuspend cell pellet in 300 µL of buffer.
10. Add 150 µL of Fix Solution and 5 µL of Discriminator Stop Reagent to each tube.
11. Proceed to flow cytometric analysis.

2.2 Immunofluorescent staining and lysis of whole blood

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O), for example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 mL of ddH_2O.

   ▲ **Note:** Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.
2. Label two tubes with A (sample) or B (control).
3. Carefully pipette 300 µL of whole anticoagulated blood to the bottom of each tube.
4. Add 20 µL BDC Phenotyping Cocktail, anti-human to tube A and 20 µL BDC Control Cocktail, anti-human to tube B.
5. Add 10 µL Dead Cell Discriminator to each tube.
6. Mix gently and incubate for 10 minutes in a horizontal position on ice under a light source (distance 3–5 cm).

   ▲ **Note:** Dead Cell Discriminator is a fluorescent photolytic dye that needs exposure to light for irreversible binding. Refer to the data sheet of the Fixation and Dead Cell Discrimination Kit (# 130-091-163) for more information.
7. Add 4 mL of 1× Red Blood Cell Lysis Solution.
8. Mix gently and incubate for 10 minutes in the dark at room temperature.
9. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
10. Resuspend cell pellet in 4 mL of buffer.
11. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
12. Resuspend cell pellet in 300 µL of buffer.
13. Add 150 µL of Fix Solution and 5 µL of Discriminator Stop Reagent to each tube.
14. Proceed to flow cytometric analysis.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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