Miltenyi Biotec

8-Color Immunophenotyping Kit
human

Order no. 130-120-640

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

0.5 mL 8-Color Immunophenotyping Cocktail, human:
Cocktail of fluorochrome-conjugated recombinant engineered REAfinity™ Antibodies:
CD3 conjugated to PE (clone: REA613, isotype: recombinant human IgG1),
CD4 conjugated to Vio® Bright 667 (clone: REA623, isotype: recombinant human IgG1),
CD8 conjugated to APC-Vio 770 (clone: REA734, isotype: recombinant human IgG1),
CD14 conjugated to VioBlue® (clone: REA599, isotype: recombinant human IgG1),
CD16 conjugated to Vio Bright 515 (clone: REA423, isotype: recombinant human IgG1),
CD19 conjugated to PE-Vio 770 (clone: REA675, isotype: recombinant human IgG1),
CD45 conjugated to VioGreen™ (clone: REA747, isotype: recombinant human IgG1),
CD56 conjugated to Vio Bright 515 (clone: REA196, isotype: recombinant human IgG1).

0.1 mL CD4-Vio Bright 667, human:
Recombinant engineered CD4 antibodies conjugated to Vio Bright 667 (clone: REA623, isotype: recombinant human IgG1) for compensation control.

0.1 mL CD8-APC-Vio 770, human:
Recombinant engineered CD8 antibodies conjugated to APC-Vio 770 (clone: REA734, isotype: recombinant human IgG1) for compensation control.

0.1 mL CD19-PE-Vio 770, human:
Recombinant engineered CD19 antibodies conjugated to PE-Vio 770 (clone: REA675, isotype: recombinant human IgG1) for compensation control.

0.1 mL CD56-Vio Bright 515, human:
Recombinant engineered CD56 antibodies conjugated to Vio Bright 515 (clone: REA196, isotype: recombinant human IgG1) for compensation control.

0.5 mL 7-AAD Staining Solution for the optional exclusion of dead and apoptotic cells from flow cytometric analysis.

10 mL 10× Red Blood Cell Lysis Solution

Capacity 50 tests or up to 5×10^8 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.

1.1 Background information

The 8-Color Immunophenotyping Kit simplifies the flow cytometric evaluation of cell fractions for immunofluorescent staining of whole blood, peripheral mononuclear cells (PBMCs), lysed whole blood samples, or other single-cell suspensions from human tissue.
The kit cocktail has been designed for the reliable identification of human monocytes, neutrophils, eosinophils, and T, B, and NK lymphocyte populations as well as $CD4^+$, $CD8^+$, and $CD56^+CD3^+$ T cell subsets in human blood.
For flow cytometric analysis use a flow cytometer equipped with a red (638 nm), a blue (488 nm), and a violet (405 nm) laser, for example, the MACSQuant® Analyzer 10.

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1.2 Applications

- Evaluation of leukocyte subsets in whole blood, PBMCs, lysed whole blood, or other single-cell suspensions from human tissue.

1.3 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.

- Flow cytometer, e.g., MACSQuant® Analyzer 10 (# 130-096-343)

  ▲ **Note:** The MACSQuant VYB cannot be used.

- (Optional) MACS MiniSampler Plus (# 130-105-745)
- (Optional) Chill 5 Rack (# 130-092-951)
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693), anti-mouse Igκ (# 130-097-900), or anti-human Igκ (# 130-104-187), for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocols

2.1 Manual immunofluorescent staining of nucleated cells, e.g., PBMCs

▲ Volumes given below are for **up to 10^7** 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 2×10^7 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 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the 8-Color Immunophenotyping Cocktail.
5. (Optional) Add 1 µL of the 7-AAD Staining Solution.
6. 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.

7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

   ▲ **Note:** Store samples at 2–8 °C protected from light until analysis.

9. Proceed to flow cytometric analysis.

2.2 Immunofluorescent staining and lysis of whole blood (lyse/no wash)

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. Add 10 µL 8-Color Immunophenotyping Cocktail per 100 µL of whole blood.
3. (Optional) Add 10 µL of the 7-AAD Staining Solution.
4. Mix well and incubate for 10 minutes in the dark at room temperature.

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

5. Add 2 mL of 1× Red Blood Cell Lysis Solution and immediately vortex thoroughly for 3 seconds. Incubate for 15 minutes in the dark at room temperature.
6. Proceed immediately to flow cytometric analysis.

2.3 Immunofluorescent staining and lysis of whole blood (lyse/wash)

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. Add 10 µL 8-Color Immunophenotyping Cocktail per 100 µL of whole blood.
3. (Optional) Add 10 µL of the 7-AAD Staining Solution.
4. Mix well and incubate for 10 minutes in the dark at room temperature.

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

5. Add 2 mL of 1× Red Blood Cell Lysis Solution and immediately vortex thoroughly for 3 seconds. Incubate for 15 minutes in the dark at room temperature.
6. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

   ▲ **Note:** Store samples at 2–8 °C protected from light until analysis.

8. Proceed immediately to flow cytometric analysis.

2.4 Immunofluorescent staining of whole blood (no lyse/no wash)

1. Add 100 µL of buffer to 100 µL whole blood.
2. Add 10 µL 8-Color Immunophenotyping Cocktail per 200 µL total volume.
3. (Optional) Add 10 µL of the 7-AAD Staining Solution.
4. Mix well and incubate for 10 minutes in the dark at room temperature.

   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

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5. Add 3.8 mL of buffer and mix well.

6. Proceed immediately to flow cytometric analysis.
   Use the flow rate "low" for acquisition and an uptake volume of at least 200 µL to ensure a sufficient number of cells for data analysis.

2.5 Flow cytometric data acquisition with the MACSQuant® Analyzer 10 using the analysis template

▲ Please refer to the MACSQuant® Instrument user manual and software guide for detailed information on using the MACSQuant Analyzer 10.

▲ The analysis template is available on the product page of the 8-Color Immunophenotyping Kit at www.miltenyibiotec.com/130-120-640.

1. Prepare and prime the MACSQuant Analyzer. Make sure the calibration and instrument settings of the instrument have been optimized for acquisition for the 8-Color Immunophenotyping Cocktail.
   For Vio Bright 515, Vio Bright 667, PE-Vio 770, and APC-Vio 770 compensation use CD56-Vio Bright 515, CD4-Vio Bright 667, CD19-PE-Vio 770, and CD8-APC-Vio 770 provided with the kit

2. Import and load the analysis template "8-Color Immunophenotyping Kit".

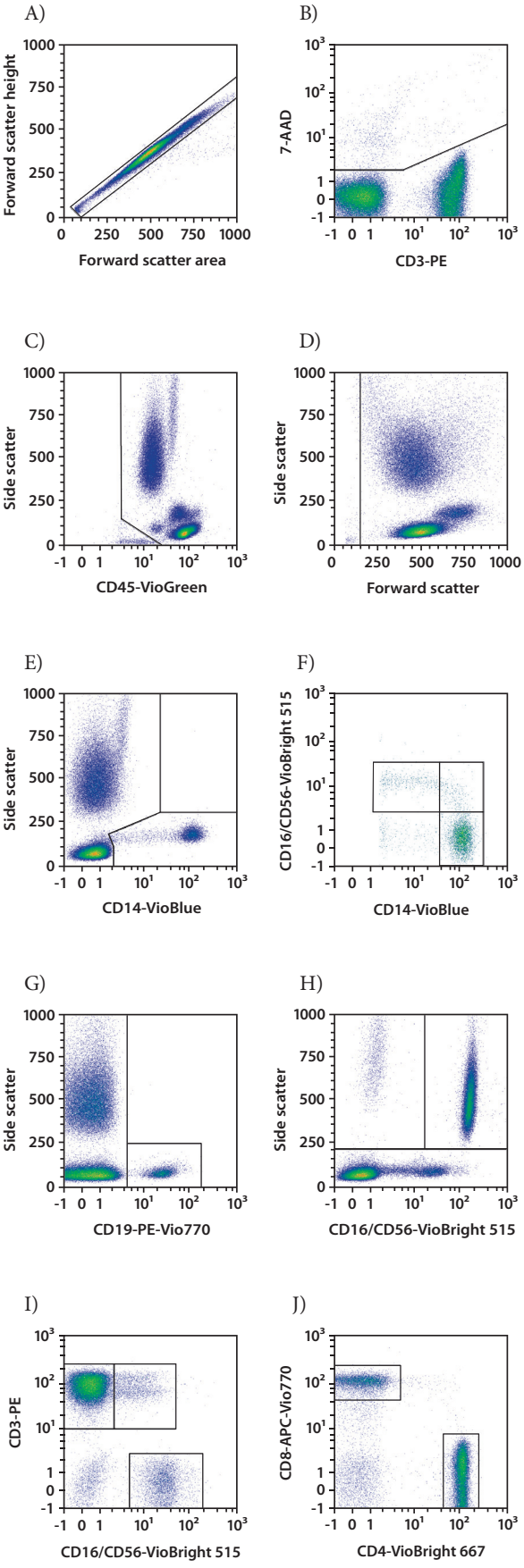
3. For doublets discrimination choose **Height**. Therefore click the **Advanced** button located in the **Channels** tab and click on the **Height** button.

4. Define an appropriate threshold, based on CD45-VioGreen versus side scatter (SSC), for the exclusion of debris and erythrocytes from the data acquisition

5. Start flow cytometric data acquisition.

3. Examples of immunofluorescent staining with the 8-Color Immunophenotyping Kit

Whole blood from a healthy donor was stained with the 8-Color Immunophenotyping Kit, human. Staining was carried out at room temperature for 10 minutes. Subsequently, red blood cells were lysed by incubation using 1× Red Blood Cell Lysis Solution at room temperature for 15 minutes. Cells were analyzed by flow cytometry using the MACSQuant Analyzer 10.
As a preliminary step for elimination of doublets a gate around single cells in forward scatter area (FSC-A) versus forward scatter height (FSC-H) (A) as well as a gate around viable cells was set (B). To identify the major circulating blood cell types CD45 was used to target all leukocytes (C). Theses cells were further separated from debris via forward scatter (FSC) and side scatter (SSC) (D). Monocytes were discriminated based on their CD14 expression (E) and then further divided into classical, intermediate, and non-classical monocytes via CD16 (F). Among the non-monocyte population, B cells were defined as $CD19^+$ (G). The remaining cells were separated into $CD16^+/SSC^{high}$ neutrophils, $CD16^-/SSC^{high}$ eosinophils as well as a $CD16^{-/dim}/SSC^{low}$ population (H). CD3 and CD56 were used to distinguish $CD56^+$ NK cells, $CD3^+$ T cells and a $CD3^+CD56^+$ T cell population (I). The T cells were divided into $CD4^+$ and $CD8^+$ T cells (J).



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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