Miltenyi Biotec

7-Color Immunophenotyping Kit
human

Order no. 130-098-456

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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

0.5 mL 7-Color Immunophenotyping Cocktail, human:
Cocktail of fluorochrome-conjugated monoclonal antibodies:
CD14 antibody conjugated to FITC (clone: Tük4, isotype: mouse IgG2a),
CD56 conjugated to PE (clone: REA196, isotype: recombinant human IgG1),
CD16 conjugated to PE (clone: REA423, isotype: recombinant human IgG1),
CD4 conjugated to PerCP (clone: VIT4, isotype: mouse IgG2a),
CD19 conjugated to PE-Vio® 770 (clone: LT19, isotype: mouse IgG1),
CD3 conjugated to APC (clone: BW264/56, isotype: mouse IgG2a),
CD8 conjugated to APC-Vio 770 (clone: BW135/80, isotype: mouse IgG2a),
CD45 conjugated to VioBlue® (clone: 5B1, isotype: mouse IgG2a).

0.1 mL CD19-PE-Vio 770:
Monoclonal CD19 antibodies conjugated to PE-Vio770 for compensation control.

0.1 mL CD8-APC-Vio 770:
Monoclonal CD8 antibodies conjugated to APC-Vio770 for compensation control.

10 mL 10× Red Blood 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 7-Color Immunophenotyping Kit simplifies the flow cytometric evaluation of cell fractions for automated and manual immunofluorescent staining of whole blood, peripheral mononuclear cells (PBMCs), or lysed whole blood samples.
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 red (638 nm), blue (488 nm), and violet (405 nm) laser, for example, the MACSQuant® Analyzer 10.
The fully automated flow cytometric analysis with the MACSQuant Analyzer 10 using the ExpressMode Immunophenotyping_7_Color_Kit_h will identify automatically the target population and gives a calculation of the frequency of target populations.

1.2 Applications

- Evaluation of leukocyte subsets in PBMCs, whole blood, or lysed whole blood.

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.

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- (Optional) MACS MiniSampler Plus (# 130-105-745)
- (Optional) Chill 5 Rack (# 130-092-951)
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

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 7-Color Immunophenotyping Cocktail.
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 per 10^7 cells and 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 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 7-Color Immunophenotyping Cocktail per 100 µL of whole blood.
3. 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.
4. 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.
5. Proceed immediately to flow cytometric analysis.

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

1. Add 100 µL of buffer to 100 µL whole blood.
2. Add 10 µL 7-Color Immunophenotyping Cocktail per 200 µL total volume.
3. 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.
4. Add 3.8 mL of buffer and mix well.
5. 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.

2.4 Automated immunofluorescent staining of whole blood and flow cytometric data acquisition with the MACSQuant® Analyzer 10 using analysis templates

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

▲ Analysis templates are available on the product page of the 7-Color Immunophenotyping Kit at www.miltenyibiotec.com/130-098-456.

1. Prepare and prime the MACSQuant Analyzer. Make sure the calibration and instrument settings of the instrument have been optimized for acquisition.
2. Pipette 100 µL of whole blood into a 5 mL round bottom tube and place it into a Chill 5 Rack on the MACS® MiniSampler Plus.
3. Define an appropiate trigger, based on CD45-VioBlue® versus side scatter (SSC), for the exclusion of debris and remaining erythrocytes from the data acquisition.
4. Click on the Barcode reader icon. Position the barcode label of the 7-Color Immunophenotyping Cocktail vial in front of the barcode reader. Place the vial into the Chill 5 Rack.
5. Assign your reagents under the **Autolabel** tab.
6. Define your sample position and ID on the Chill 5 Rack.
7. Import and load the analysis template "7-Color Immunophenotyping Kit_No lysis".
8. Start automated immunofluorescent staining and flow cytometric data acquisition.

2.5 Fully automated flow analysis with the MACSQuant® Analyzer 10 using the Express Mode

▲ For fully automated flow cytometric analysis of PBMCs, whole blood, or lysed whole blood with the MACSQuant® Analyzer 10, use the 7-Color Immunophenotyping Express Mode function. For details of how to use the Express Modes, please refer to the MACSQuant Instrument user manual, the MACSQuantify™ Software guide, or visit www.macsquant.com.

By selecting the Express Mode all experiment settings are automatically loaded. The loaded values are shown in the respective fields in the **Experiment** tab. These can be adapted manually if needed, for example, uptake volume.

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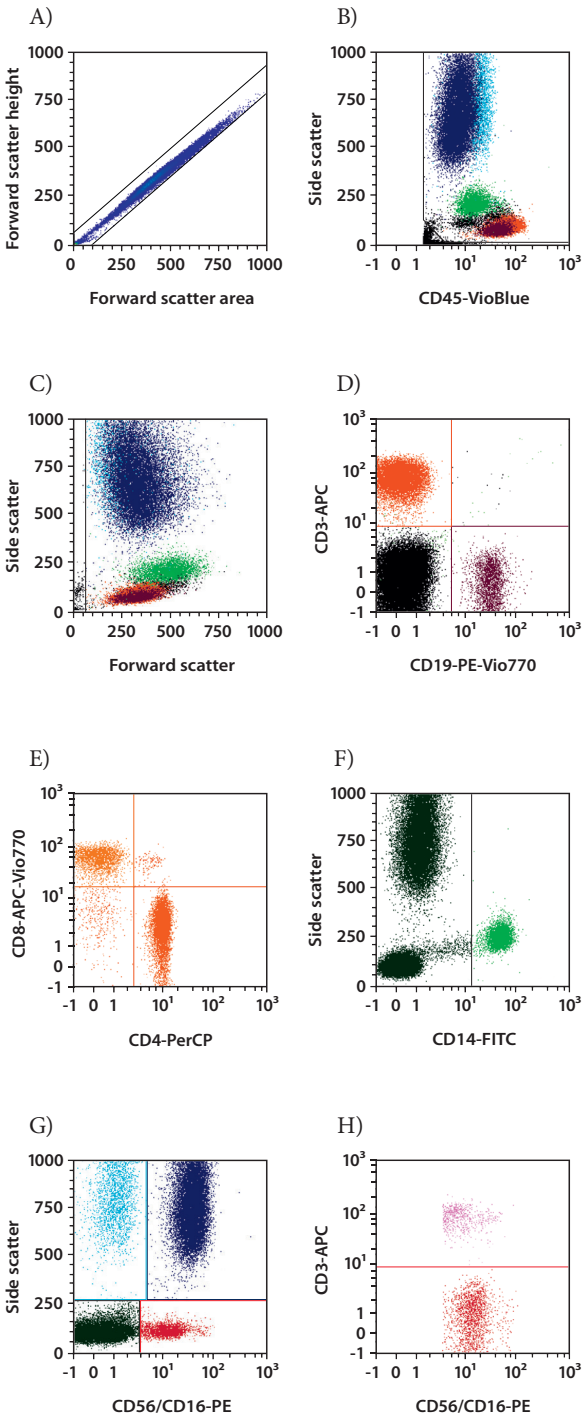
1. Prepare and prime the MACSQuant Analyzer 10. Make sure the calibration and compensation settings have been optimized for acquisition of the 7-Color Immunophenotyping Cocktail. For PE-Vio® 770 and APC-Vio 770 compensation use CD19-PE-Vio 770 and CD8-APC-Vio 770 within the kit.
   The setting must be adapted to the applied cell material
2. For doublets discrimination choose **Height**. Therefore click the **Advanced** button located in the **Channels** tab and click on the **Height** button.
3. For automated gating using the Express Mode choose an optimal forward scatter area (FSC-A) voltage, so that the population is positioned between 250–500 for linear scale.
4. Define an appropriate trigger, based on CD45-VioBlue® versus side scatter (SSC), for the exclusion of debris and erythrocytes from the data acquisition. To select the channel for the trigger, click the **Channel** tab and choose the **UV V1 Trigger**.
5. Load the Express Mode by selecting the **Settings** tab
6. Check the **Express** button.
7. Select **Analysis** from the **Type** drop-down list.
8. Choose the Express Mode **Immunophenotyping_7_Color_Kit_h** from the **Mode** drop-down list.
   ▲ **Note:** Optimal Express Mode analysis requires hlog setting for the fluorochrome channels and activated Height parameter. These two parameters will be set automatically by the Express Mode and should not be changed.
9. Start the measurement.
10. For the analysis of data files right-click within the **Samples** tab and select **Open...** or **Add…** from the context menu to add data files to the MACSQuantify™ Software.
11. Navigate to desired data files, select them, and open them into the software.
12. Right-click on the file name and select **View with express Analysis. Immunophenotyping_7_Color_Kit_h** for accessing the Express Mode analysis template. The data will be displayed in an analysis window.
    ▲ **Note:** Gate A (FSC-H versus FSC-A) and gate G (SSC versus CD56/CD16-PE) may require manual adjustment.
13. The selection has to be repeated for each analyzed file.

4. Examples of immunofluorescent staining with the 7-Color Immunophenotyping Kit

Whole blood from a healthy donor was stained with the 7-Color Immunophenotyping Kit, human. Staining was carried out at 4 °C for 10 minutes. Subsequently, red blood cells were lysed by incubation using 1× Red Blood Cell Lysis Solution at room temperature for 15 minutes and analyzed by flow cytometry using the Express Mode of the MACSQuant® Analyzer.
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) was set (A). To identify the major circulating blood cell types CD45 was used to target all leukocytes (B). Theses cells were further separated from debris via forward scatter (FSC) and side scatter (SSC) (C). CD19 has been used to define B cells and CD3 for T cells (D). The T cells were separated in $CD4^+$ and $CD8^+$ T cells (E). CD14/SSC intermediate were selected to identify monocytes (F). Monocytes were excluded and the remaining cells were further characterized. A gate was defined on CD16/SSC^{high} cells to identify mature neutrophils, which could be separated from eosinophils due to the absence of CD16 expression. A CD16/CD56/SSC^{low} gate was used for sub-characterization (G). These cells were further characterized by gating on CD3/$CD56^+$ T cells and CD56/$CD16^+$ NK cells (H).



Statistic information:

7-Color Immunophenotyping Kit, h

EM 1.1408

Sample ID: 165
Description: lyse/no wash
File Name: _srv2013-01-07_2367.010

Cell type	Gating strategy	Color	Cells/µL in measurement	[%] among leukocytes
Leukocytes	$CD45^+$		194.68	
T cells	$CD45^+$. $CD3^+$		49.57	25.46
$CD4^+$	$CD45^+$. $CD3^+$. $CD4^+$		34.28	17.61
$CD8^+$	$CD45^+$. $CD3^+$. $CD8^+$		12.90	6.63
B cells	$CD45^+$. $CD19^+$		12.84	6.60
Monocytes	$CD45^+$. $CD14^+$		18.72	9.62
NK cells	SSC^{low},$CD45^+$,$CD14^-$,$CD16^+$,$CD56^+$,$CD3^-$		9.25	4.75
$CD3^+$ $CD56^+$	SSC^{low},$CD45^+$,$CD14^-$,$CD16^+$,$CD56^+$,$CD3^+$		2.98	1.53
Eosinophils	SSC^{high}. $CD45^+$. $CD14^-$. $CD16^-$		12.76	6.56
Neutrophils	SSC^{high}. $CD45^+$. $CD14^-$. $CD16^+$		85.15	43.74

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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