

7-Color Immunophenotyping Kit, human

Immunophenotyping as easy as it gets

Introduction

The challenge

Flow cytometric immunophenotyping and enumeration of a comprehensive range of immune cell subsets can be a tedious challenge. Establishing an appropriate assay usually requires laborious antibody titration, and the assay itself involves numerous pipetting steps and a complex flow cytometric analysis. A large number of manual handling steps can limit the level of reproducibility and thus the quality of the results.

The solution

Miltenyi Biotec has developed the 7-Color-Immunophenotyping Kit, which simplifies all steps from fluorescent labeling to multicolor flow cytometric immunophenotyping. This kit enables the simultaneous enumeration of monocytes, neutrophils, eosinophils, and T, B, and NK cells, as well as helper/inducer T cells, cytotoxic/suppressor T cells, and CD3⁺CD56⁺ cells.

Whole blood samples can be processed optionally with the enclosed Red Blood Cell Lysis Solution in a single lysis step without washing. All antibodies are pre-titrated and combined in a single ready-to-use cocktail for optimal, reproducible staining of peripheral blood mononuclear cells (PBMCs), whole blood, or lysed whole blood.

The 7-Color-Immunophenotyping Kit in combination with a MACSQuant[®] Flow Cytometer enables fully automated fluorescent labeling, compensation, and analysis. Nevertheless, users of other flow cytometers can also take advantage of the kit's benefits.

Method

The 7-Color-Immunophenotyping Kit includes a cocktail of monoclonal fluorochrome-conjugated antibodies: CD3-APC, CD4-PerCP, CD8-APC-Vio[®] 770, CD14-FITC, CD16-PE, CD19-PE-Vio770, CD45-VioBlue[®], and CD56-PE. Additionally, pre-titrated CD19-PE-Vio770 and CD8-APC-Vio770 antibodies are included for accurate compensation control. The Red Blood Cell Lysis Solution allows for simple processing of whole blood samples.

In brief, the procedure consists of three steps:

1. Fluorescent labeling with the 7-Color Immunophenotyping Cocktail.
2. Red blood cell lysis (optional).
3. Cell analysis by flow cytometry.

Using the MACSQuant Flow Cytometers whole blood samples can be processed fully automatically from fluorescent labeling to multicolor analysis, without red blood cell lysis.

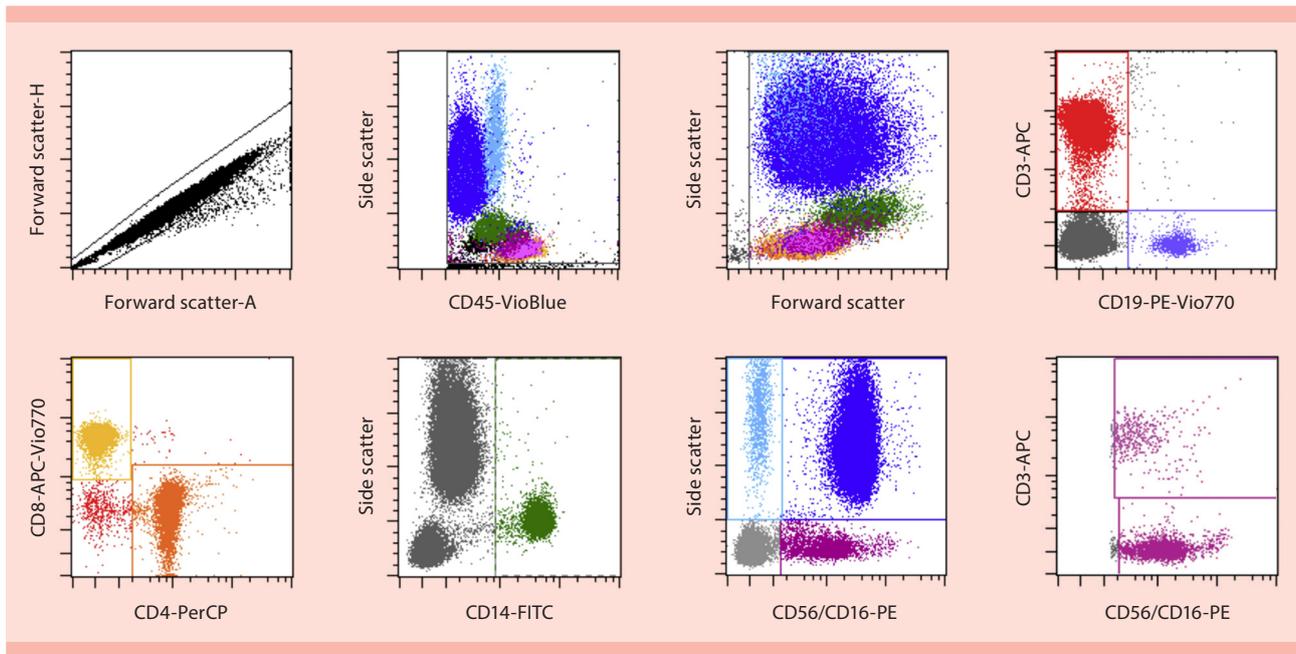
Using the MACSQuant Flow Cytometer together with the Express Mode of the MACSQuantify[™] Software allows the automated identification of the target population including a calculation of the frequency.

For a detailed, easy-to-follow protocol, refer to the data sheet available at www.miltenyibiotec.com/130-098-456

Results

Figure 1 shows a typical result of the enumeration of leukocyte subsets from whole blood of a healthy donor. Cells were analyzed on the MACSQuant Analyzer according to the gating strategies shown in the table.

Product	Order no.
7-Color Immunophenotyping Kit, human	130-098-456



Cell type	Gating strategy	Color	Cells/ μ L	% among leukocytes
Leukocytes	CD45 ⁺	Grey	5.51×10^3	100
T cells	CD45 ⁺ , CD3 ⁺	Red	1.21×10^3	22 (22.47 \pm 3.84)
CD4 ⁺ T cells	CD45 ⁺ , CD3 ⁺ , CD4 ⁺	Orange	8.08×10^2	15 (14.55 \pm 3.51)
CD8 ⁺ T cells	CD45 ⁺ , CD3 ⁺ , CD8 ⁺	Yellow	3.33×10^2	6 (6.76 \pm 1.32)
B cells	CD45 ⁺ , CD19 ⁺	Blue	1.32×10^2	2.4 (5.16 \pm 2.32)
Monocytes	CD45 ⁺ , CD14 ⁺	Green	4.19×10^2	7.6 (8.41 \pm 1.32)
NK cells	SSC ^{low} , CD45 ⁺ , CD14 ⁻ , CD16 ⁺ , CD56 ⁺ , CD3 ⁻	Purple	2.84×10^2	5.2 (4.39 \pm 2.42)
CD3 ⁺ CD56 ⁺ cells	SSC ^{low} , CD45 ⁺ , CD14 ⁻ , CD56 ⁺ , CD16 ⁺ , CD3 ⁺	Light Purple	0.46×10^2	0.84 (0.78 \pm 0.67)
Eosinophils	SSC ^{high} , CD45 ⁺ , CD14 ⁻ , CD16 ⁻	Cyan	1.96×10^2	3.6 (3.21 \pm 1.59)
Neutrophils	SSC ^{high} , CD45 ⁺ , CD14 ⁻ , CD16 ⁺	Dark Blue	3.23×10^3	59 (53.78 \pm 6.12)

Figure 1: Gating strategies for the enumeration of multiple leukocyte subsets. Whole blood from a healthy donor was stained using the 7-Color Immunophenotyping Kit. Staining was carried out at 4 °C for 10 minutes. Subsequently, red blood cells were lysed by incubation with Red Blood Cell Lysis Solution at 4 °C for 15 minutes. Cells were analyzed using the MACSQuant Analyzer. Absolute cell numbers (cells/ μ L) and individual cell percentages (% among leukocytes) are from the illustrated experiment. Frequencies in parentheses indicate means \pm sd from nine independent experiments.

The 7-Color Immunophenotyping Kit in a nutshell

- Simultaneous enumeration of **pan T cells, CD4⁺ T cells, CD8⁺ T cells, CD3⁺CD56⁺ cells, B cells, NK cells, monocytes, eosinophils, and neutrophils**
- Single antibody cocktail, reduced hands-on time, excellent reproducibility
- Pre-titrated and ready-to-use antibody cocktail
- All cell types are enumerated from a single sample, saving tubes and reagents, and thus costs
- Red Blood Cell Lysis Solution included for an optional quick lysis, no-wash procedure
- Compensation reagents included for accurate analysis of tandem dyes
- Fully automated analysis on the MACSQuant® Flow Cytometers using optimized gating strategies
- Minimized manual handling increases user safety



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