Flow Cytometry for Monocyte Phenotype and Mitochondrial Mass

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Abstract

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Materials

- K2EDTA Vacutainer Tubes 366643 by Bd
- 12x75mm test tubes 0555512 by Fisher Scientific
- High-Yield Lysing Buffer HYL250 by Thermo Fisher Scientific
- Staining Buffer 554656 by BD Biosciences
- CD14-PE Antibody 561707 by BD Biosciences
- CD16-BV421 Antibody 562878 by BD Biosciences
- MitoTracker Green FM 9047S by Cell Signaling Technology
- IgG From Human Serum I8640 by Sigma
- ✓ Pipettes and P1000, P200, P10 tips by Contributed by users

Protocol

Step 1.

Materials to Prepare

Mitotracker Green FM

Prepare as directed and store 1mM stock solution at -20°C.

Step 2.

Collect blood in K2EDTA tube and store at room temperature until use.

Step 3.

Aliquot 100 µl whole blood into 12×75mm test tubes, 1 tube per subject.

Step 4.

Aliquot blood into control tubes (can use blood from one subject or mixed blood, 100 µl per tube)

(a) Unstained control

(b) CD14 only control

(c) CD16 only control

(d) Mitotracker only control

(e) FMO (note 1) no CD14

(f) FMO no CD16

(g) FMO no Mitotracker

NOTES

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FMO = fluorescence minus one control. Includes all stains except the one indicated (e.g. FMO no CD16 includes CD14 and Mitotracker stains, but not CD16 stain).

Step 5.
Add 10 µg IgG to each tube and incubate 15 minutes at 4degC-25degC to block Fc receptors

Step 6.
Add antibodies to tubes:CD14 antibody: 20 µl to all samples plus CD14 only control, FMO no CD16, FMO no MitotrackerCD16 antibody: 1 µl to all samples plus CD16 only control, FMO no CD14, FMO no Mitotracker

Step 7.
Vortex gently and incubate 1 hour in the dark at 4degC-25degC

Step 8.
Add 1 ml of lysing buffer, vortex gently and incubate in the dark at 4degC-25degC for 10 minutes.

Step 9.
While lysing, prepare MitotrackerDilute 1 µl stock in 999 µl stain bufferDilute 200 µl initial dilution in 800 µl stain buffer for final concentration of 200 nM

Step 10.
Centrifuge lysed blood at 500×g for 5 minutes. Remove supernatant.

Step 11.
Add 200 µl Mitotracker to all samples plus Mitotracker only control, FMO no CD14, FMO no CD16Add 200 µl staining buffer to unstained control, CD14 only, CD16 only, FMO no Mitotracker.

Step 12.
Incubate 1 hour in the dark at 4degC-25degC.

Step 13.
Add 1 ml staining buffer and centrifuge 500×g for 5 minutes. Remove supernatant.

Step 14.
Repeat step 12.

Step 15.

Resuspend in 400 µl lysing buffer. Analyze as quickly as possible.

Step 16.

Data Analysis

For the listed antibodies, a flow cytometer with blue (488 nm) and violet (405 nm) lasers is necessary. Adjust forward scatter, side scatter, and fluorescence detector sensitivity as appropriate.

Gate based on unstained control and FMO controls with compensation based on single stain tubes.

Gate monocyte population (**Figure 1A**) and show scatter plot of CD16 vs. CD14 (**Figure 1B**). Mitotracker data can be analyzed as median fluorescence intensity between samples or cell populations.

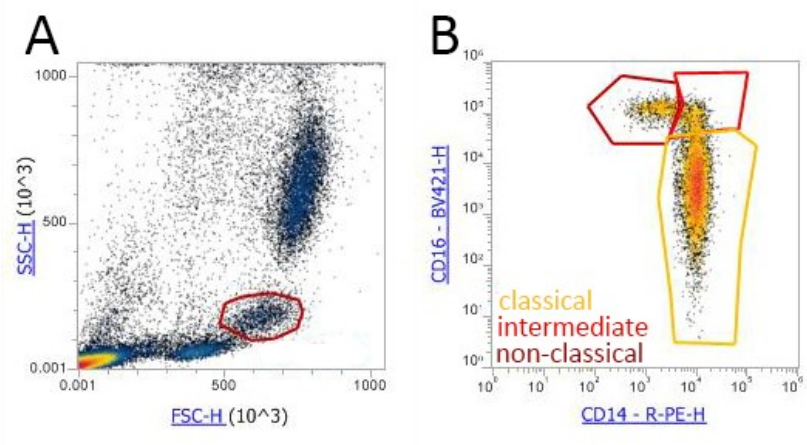

Figure 1. Typical flow cytometry results. (A) Typical forward and side scatter plot. Monocytes are gated. (B) Typical CD16 vs. CD14 plot. Monocytes are separated into classical (CD14+CD16-), intermediate (CD14+CD16+), and non-classical (CD14dimCD16+) subtypes.