



6 Color Panel for Staining Human Peripheral Blood Lymphocytes

Introduction

Here we describe immunophenotypic analysis of human peripheral blood lymphocytes utilizing a 6-color antibody panel in a stain/lyse protocol run on the CytoFLEX* Research Flow Cytometer. The markers chosen are commonly used surface CD antigens which can be used to quantify and characterize normal and abnormal lymphocyte populations in peripheral blood.

The CytoFLEX is excellent for immunophenotyping research experiments. The proprietary Wavelength Division Multiplexing (WDM) detection module uses solid-state, high efficiency, low-noise Fiber Array Photodiode Detectors (FAPD), giving exceptional resolution for more precise data and better detection of rare events. This protocol describes the use of the CytoFLEX with a red and blue laser configuration for six-color immunophenotyping analysis.

Materials and tools

1. Human peripheral blood sample drawn in EDTA tube
 - a. used within 24 hours of draw
2. Hypotonic Lysing Solution
3. Phosphate Buffered Saline (PBS)
4. Deionized water
5. Vortex
6. Centrifuge
7. 12x75mm tubes
8. Pipettes and tips
9. CytoFLEX flow cytometer - red and blue laser configuration

488nm		
FITC	PE	PerCP
CD3	CD16/56	CD45
638nm		
Cy7	APC	APC-Cy7
CD4	CD19	CD8

Table 1: Antibody Cocktail

Sample Preparation

1. Add 50µL mixed blood sample to antibody cocktail in a 12 X 75 mm test tube.
2. To prepare cocktail from single color antibody stocks, refer to the package instructions; antibody titration is recommended.
3. Vortex the tube gently to mix.
4. Incubate the sample at room temperature in dark for 15 minutes.
5. Add Lysing Solution per package instructions and vortex gently.
6. Incubate the tube per package instructions in dark.
7. Centrifuge the sample tube at 1500rpm for 5 minutes.
8. Discard the supernatant, add 1mL PBS and vortex gently.
9. Centrifuge the sample tube at 1500rpm for 5 minutes again.

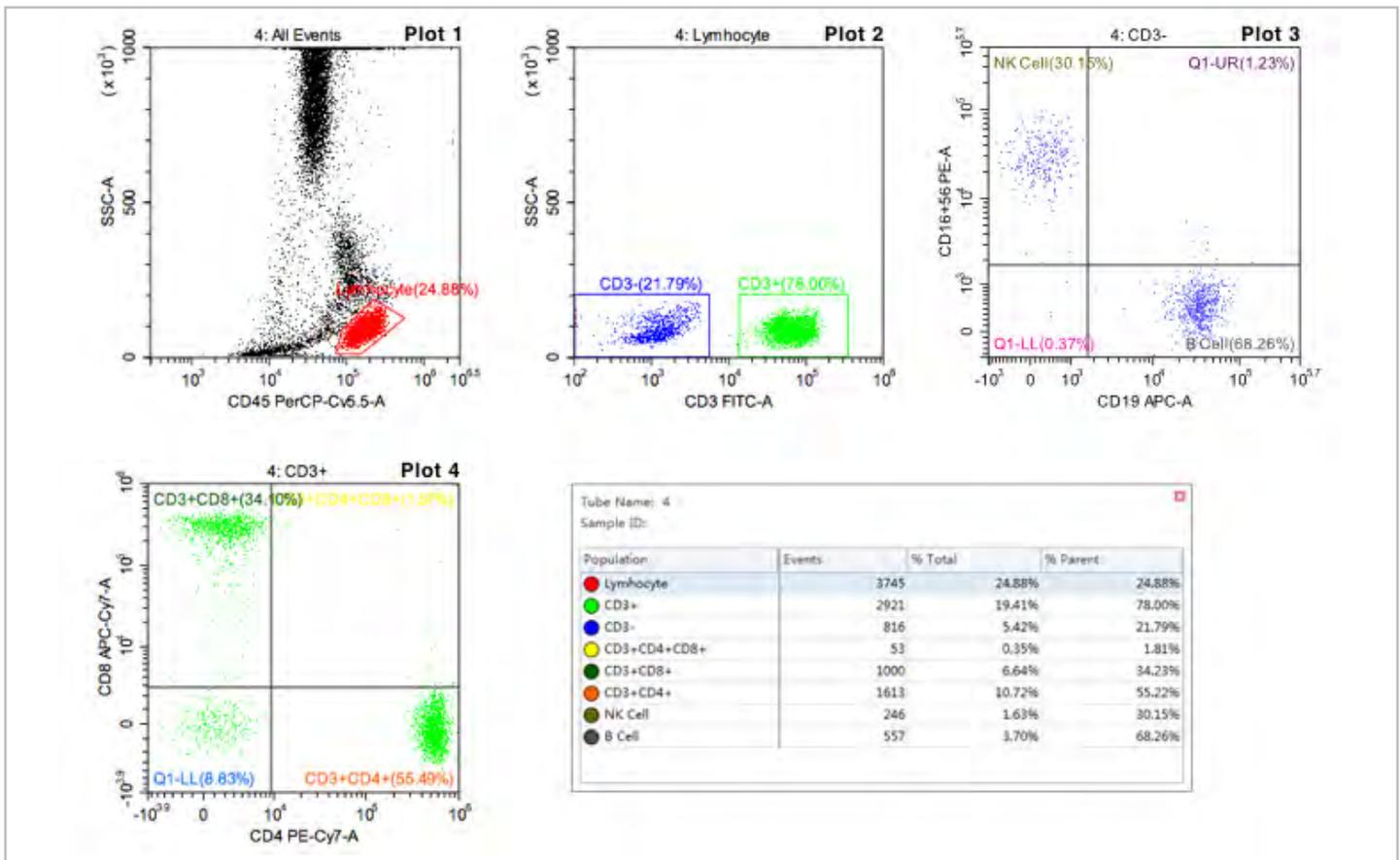


Figure 1: Plot 1 CD45 PerCP Cy5.5 vs SSC showing the positioning of the lymphocyte gate.

Sample Preparation (cont'd.)

10. Discard the supernatant and add 500µL PBS.
11. Vortex the sample tubes gently. Store tubes at room temperature in dark; perform analysis within 1 hour.

Data Acquisition and Analysis

1. Run the sample at low flow rate setting.
2. Set the threshold on PerCP-Cy5.5 channel to cut off the debris.
3. Adjust the gains and compensation, if needed.
4. Collect 15,000 events.
5. Set the lymphocyte gate on CD45 bright/SSC low population.
6. Use "Fit with Sample" function to show the low signals.

Conclusions

Here is a demonstration of a simple six color analysis of human blood peripheral blood lymphocytes using the CytoFlex flow cytometer. There is clear delineation of each of the populations of interest.



Beckman Coulter | 11800 SW 147th Avenue, Miami, FL 33196 | 305.380.3800 | www.beckmancoulter.com

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