



FLOW CYTOMETRY

# CyAn™ ADP | Dendritic Cells: Rare Event Analysis

Dendritic cells are of great interest due to their ability to stimulate and regulate immune response. Studying these cells has become increasingly important for research in cancer, AIDS, arthritis, diabetes or other immune abnormalities. Their antigen presentation capability enables the development of vaccines and immune therapies. However, they are sometimes difficult to study since they are a complex, rare event population which cannot be identified by a single marker. This protocol demonstrates an easy way to identify and isolate both CD11c+ and CD123+ dendritic cell subsets on a CyAn ADP LX 9 Color flow cytometer.

## Materials and Methods

A cocktail of lineage markers was prepared in one tube using 8 µL of each of the following mouse anti-human FITC-conjugated antibodies: CD3, CD14, CD16, CD19, CD20 and CD56. In two other tubes, 400 µL of whole blood collected from a normal, healthy adult was added. To one of the tubes, the following mouse anti-human antibodies were added: 20 µL of the FITC cocktail, 40 µL of HLA-DR APC and 20 µL of CD123 RPE. In the other tube, the following mouse anti-human antibodies were added: 20 µL of the FITC cocktail, 40 µL of HLA-DR APC, and 20 µL of CD11c RPE. After antibody addition, the two tubes were gently vortexed and incubated for 30 minutes in the dark at room temperature. After incubation, 800 µL of Uti-Lyse™ Reagent A was added to each tube. The tubes were gently vortexed and incubated for exactly 10 minutes in the dark at room temperature. After 10 minutes, 8 mL of Uti-Lyse Reagent B was added to each tube. The tubes were gently vortexed and incubated for 10 minutes in the dark at room temperature. After the tubes were centrifuged for 5 minutes at 300 x g, the supernatant was removed and the pellets were resuspended in 4 mL HBSS (Hank's Buffered Salt Solution with 1% BSA, 0.1% NaN<sub>3</sub>, and 1 mmol/L EDTA). The tubes were vortexed and centrifuged for 5 minutes at 300 x g. After removing the supernatant, the pellets were resuspended in 1 µL HBSS (with 4% BSA, 0.1% NaN<sub>3</sub>, and 1 mmol/L of EDTA). The tubes were stored at 4 °C until use.

## Results

The samples were analyzed on a CyAn ADP LX 9 Color. Summit software was used for gating and compensating. All PMT voltages were set so that the populations appeared on scale. Compensation was applied and the appropriate parameters displayed. Figure 1, Region 1 (R1) includes the entire leukocyte population. Figure 2 is gated on R1. Here, Region 2 (R2) excludes the bright FITC cell populations from the dim and negative populations. The dendritic cell subsets and the basophils are included within R2 in the populations negative for CD3/FITC, CD14/FITC, CD16/FITC,

CD19/FITC, CD20/FITC and CD56/FITC (FITC lineage cocktail). Figure 3 is gated on R1+R2, showing CD123+ dendritic cells (DCs), FITC<sup>dim</sup> cells and basophils. Figure 4 is gated on R1+R2, showing CD11c+ dendritic cells and FITC<sup>dim</sup> cells. Both dendritic cell subsets are positive for HLA-DR, and appear between lymphocytes and monocytes in the scatter plot.

## Discussion

As shown here, this cell preparation is an easy way to identify and isolate dendritic cells in peripheral blood. It is important to have a large enough sample to acquire a minimum of 50,000 gated events in R1 since dendritic cells typically comprise less than 1 percent of peripheral blood mononuclear cells, and often less in immunocompromised persons. The high-speed capability of the CyAn ADP LX 9 Color makes it ideal for dendritic cell analysis and other rare event applications.

Figure 1

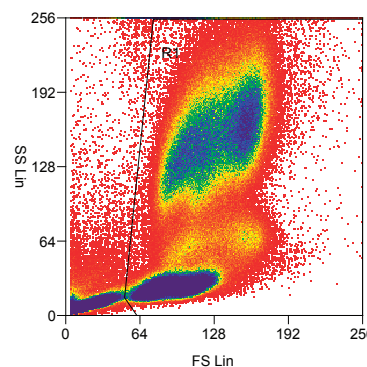


Figure 2

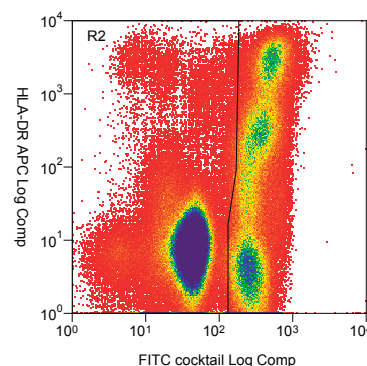


Figure 3

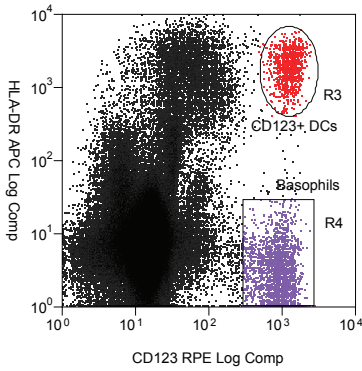


Figure 4

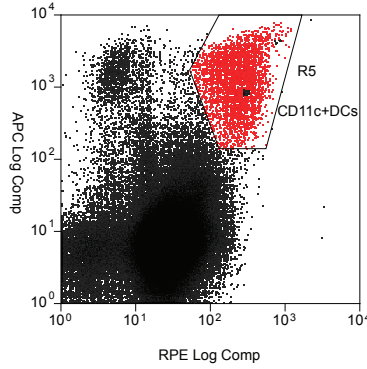
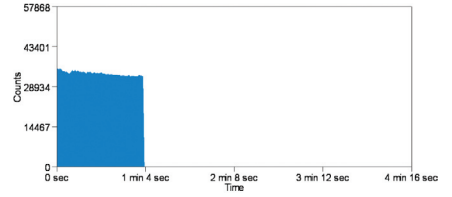


Figure 5



The acquisition rate exceeded 33,000 cells per second. This application can easily be run at high acquisition speeds. Total number of events gathered in a time period of 61 seconds = 2,056,789 total events (33,171 events per second).

## TECHNICAL TIPS

- > Blood should be used within 24 hours from collection. Stressed or old blood could cause cell activation leading to aggregation of dendritic cells with other cell populations.
- > Before running the samples, verify that the instrument has had the appropriate warm-up time.
- > During analysis, color gating can be applied to determine the various cell populations and assist with statistical analysis.
- > An isotype control reagent could be used to set PMT voltages and compensation but is not necessary since the populations are easy to distinguish and compensate using this technique.

## References

- Grouard G, Risoan M, Filgueira I, Durand I, Banchereau J, Liu J. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med.* 185:1101-1111.
- Hoffman T, Mueller-Berghaus J, Ferris R, Johnson J, Storkus W, Whiteside T. 2002. Alterations in the frequency of dendritic cell subsets in the peripheral circulation of patients with squamous cell carcinomas of the head and neck. *Clin Cancer Res.* 8: 1787-1793.
- Willmann K, Dunne JF. 2000. A flow cytometric immune function assay for human peripheral blood dendritic cells. *J Leuk Bio.* 67:536-544.

## PRODUCT

## CODE

CyAn ADP LX 9 Color .....	CY201
Monoclonal Mouse Anti-Human CD3/FITC ..	F0818
Monoclonal Mouse Anti-Human CD14/FITC ..	F0844
Monoclonal Mouse Anti-Human CD16/FITC ..	F7011
Monoclonal Mouse Anti-Human CD19/FITC ..	F0768
Monoclonal Mouse Anti-Human CD20/FITC ..	F0799
Uti-Lyse, Erythrocyte Lysing Reagent .....	S3350
Hank's Buffered Salt Solution .....	S1754

For research use only – not to be used in diagnostic procedures. Other vendor products used in this application: eBioSciences and Caltag.

The protocols in this application note might deviate from the normal recommended protocol/specification guidelines which are included with the Dako product or any other non-Dako product.