

Application Note 1

Enrichment of Monocytes for Morphological Study

Introduction

Enrichment of cell populations based on their scatter and fluorescence properties using flow cytometry and cell sorting has been described by investigators over the past two decades.¹ The FACSort™ benchtop cell sorter allows highly pure populations of cells to be sorted with a minimum setup time, while preserving the morphological characteristics of the cells. In this application note, we show that monocytes can be sorted from a lysed whole blood sample, and that cyospin preparations can be made for examination by light microscopy.*

* For research use only. Not for use in diagnostic or therapeutic procedures.

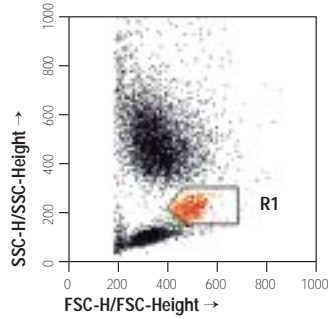


Figure 1a

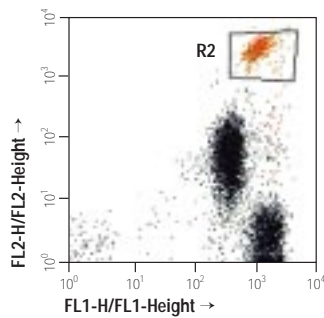


Figure 1b

Figure 1 The scatter (1a) and fluorescence (1b) profiles of a lysed whole blood preparation stained with CD45 FITC/CD14 PE.

Materials and Methods

Cells

Ten mL of whole blood was collected into a VACUTAINER® tube with EDTA anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Three 1-mL aliquots of whole blood were lysed by adding 14 mL ammonium chloride per mL of whole blood. The cells were incubated for 5 minutes at room temperature (20° to 25°C).

Staining

The cell preparations were washed twice in Ca⁺⁺/Mg⁺⁺-free Dulbecco's phosphate-buffered saline (D-PBS; BioWhittaker, Walkersville, MD), and the cell concentration was adjusted to 2 x 10⁷ cells per mL. The cells were then stained with LeucoGATE™ (CD45 FITC/CD14 PE, Becton Dickinson Immunocytometry Systems [BDIS], San Jose, CA) as described on the package insert.

The three lysed whole blood cell preparations were then pooled and resuspended in 750 µL of D-PBS at a final concentration of 4 x 10⁶ cells per mL.

Flow Cytometry

The data were acquired using the LYSYS™ II (BDIS) software provided with the FACSort. Amplifiers were set to LINEAR for scatter channels, and to LOG for the fluorescence channels. Instrument settings were optimized to resolve the major cell populations in the scatter parameters and in the fluorescence parameters. Figures 1a and 1b show the scatter and fluorescence properties of the lysed whole blood preparation, respectively. A gate was set on scatter and fluorescence to identify the monocyte cluster for sorting (G1=R1 and R2). See Figure 1.

Approximately 46,000 monocytes from the pooled, lysed whole blood cell preparations were sorted at a sort rate of approximately 30 cells per second. The cells were sorted on LO using the Single Cell mode into 50 mL tubes (Falcon®; Becton Dickinson Labware, Franklin Lakes, NJ) coated with 4% BSA (see *FACSort User's Guide*, Chapter 4). The cells were centrifuged at 250 x *g* for 5 minutes, and the supernatant was carefully aspirated. The cells were resuspended in 300 µL of D-PBS.

Cyto centrifugation and Staining

The cytospin apparatus was assembled using one filter card (Shandon Corp., Pittsburgh, PA), and a sample funnel that was coated with 0.5 mL 4% BSA-PBS for at least one hour at 4°C. The BSA-PBS was then discarded. Fetal calf serum was run through a 0.45- μm filter, and 100 μL was pipetted into the sample cup. One hundred microliters of sample was added to the sample cup and centrifuged at 700 x g for 7 minutes. The slide was allowed to air-dry for 10 minutes. Slides were then treated with Diff-Quik[®] Fixative (American Scientific Products, McGaw, IL) for 30 seconds, followed by Diff-Quik Solution I for 60 seconds, and then Diff-Quik Solution II for 60 seconds. Slides were then washed in deionized water for 30 seconds, allowed to air-dry, and mounting medium and cover slip were applied.

Results

Figure 2 shows the lysed whole blood preparation prior to sorting, stained with Diff-Quik. The monocytes comprised approximately 6% of the cell preparation, as assessed by flow cytometric analysis (data not shown). Figure 3 shows the high purity of the sorted monocytes, and the excellent retention of their morphological characteristics after sorting.

Discussion and Summary

Cells were sorted on the basis of scatter and fluorescence properties and cyto centrifuged onto a glass slide. The cells retained their morphological characteristics and were suitable for staining. Studies² have shown that as few as 1,500 to 2,000 sorted cells are enough to make a slide with more than 300 intact cells and little debris.

Hint

If you adjust the cell concentration to 10^7 cells/mL before sorting, you will approach the maximum sort rate and shorten the sort time.

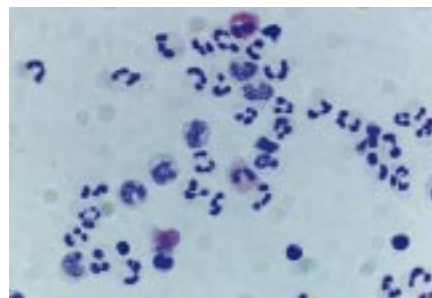


Figure 2 The lysed whole blood preparation prior to sorting and stained with Diff-Quik.

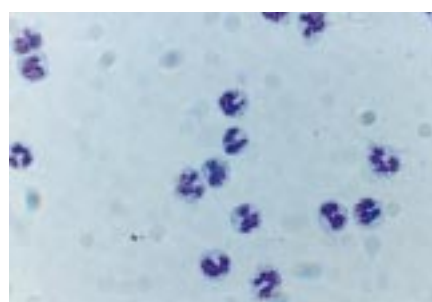


Figure 3 Monocytes sorted from lysed whole blood.

BDIS publishes this method

as a service to investigators.

Detailed support for non-flow

cytometric aspects of this

procedure may not be

available from BDIS.

References

1. Melamed MR, Mullaney PF, Shapiro HM. An historical review of the development of flow cytometers and sorters. In: Melamed MR, Lindmo T, Mendelsohn ML, eds. *Flow Cytometry and Sorting, Second Edition*. New York, New York: Wiley-Liss Inc; 1990:1-9.
2. Unpublished data. Becton Dickinson Immunocytometry Systems, 1992.

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