APPLICATION NOTE

FLOW CYTOMETRY

MoFlo® | Stem Cell Sorting

Purified populations of functional stem cells are of great interest to the biomedical community, both in the understanding of stem cell biology and in clinical transplant settings. Studies continue to define phenotypic markers, functional characteristics and in vivo reconstitutional activity for both hematopoietic and non-hematopoietic stem cells. Historically, the capture of these pluripotent cells has presented a significant laboratory challenge. In transplant programs, for instance, peripheral blood stem cells from a stimulated donor usually represent 0.01% - 1% of the collected white blood cells, and bone marrow contains 0.5% - 5% stem cells.[1] A number of strategies have emerged for the purification of these cells, including high-throughput flow cytometry based on multiparametric immunophenotyping or immunophenotying in combination with functional characteristics. The MoFlo High-Performance Cell Sorter has proved invaluable in these efforts. In the experiment shown here, the MoFlo was used to purify bone marrow side population cells.

Materials and Methods

Staining. Bone marrow from tibias and femurs of 5- to 8-week-old C57BL/6 mice was flushed into HBSS in polypropylene centrifuge tubes. The nucleated cells were enumerated in this suspension, then pelleted and resuspended at 1 x 106 cells/mL in pre-warmed DMEM. Hoechst 33342 was added to a final concentration of 5 µg/mL. Samples were mixed thoroughly and placed in a stable 37 °C water bath for exactly 90 minutes, then immediately transferred to a 4 °C centrifuge to pellet the cells. Cells were re suspended in cold HBSS and maintained at 4 °C. (If additional surface antibody labeling is desired, ensure that the cell suspension remains at 4 °C. If desired, add propidium iodide at 2 µg/mL to exclude non-viable cells from the sort.[2])

Instrument Set-up. A MoFlo with a 351 nm UV laser was configured to detect fluorescent emission both in the blue region, using a 450/20 bandpass filter, and in the red region, using a 675 eFLP filter.

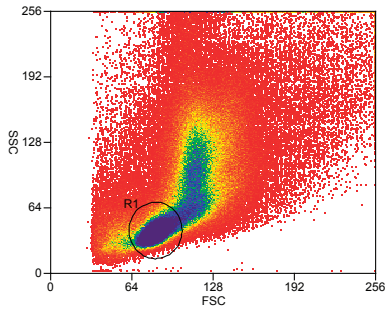
Results

Stained cells were placed on the MoFlo and a forward scatter vs. side scatter dot plot was used to gate the primary cell population (Figure 1a). A Hoechst Blue vs. Hoechst Red dot plot (Figure 1b) revealed the progenitor cells of interest, identified by their characteristic position to the left of the bulk cell population, i.e. the “side population”. These rare side population cells, a type of hematopoietic stem cells characterized by their ability to efflux Hoechst dye, were sorted for further investigation.

Discussion

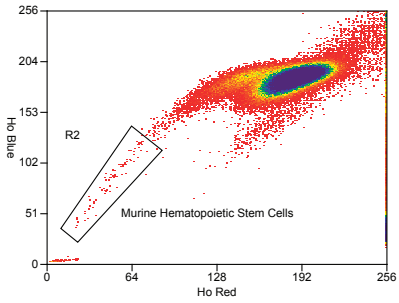
Purifying hematopoietic and non-hematopoietic stem cell populations,[3-15] including side populations,[16-27] has become routine with the MoFlo. The novel electronics, optics and fluidics of the MoFlo provide the power, precision and yield necessary to capture these rare events. Furthermore, the MoFlo uses a patented nozzle design to reduce turbulence and minimize the effects of acceleration on each cell. Thus, following purification, these cells are fully functional and capable of in vivo reconstitution, post-transplantation engraftment and long-term culture.[28-34]

Figure 1a



Plotting forward scatter vs. side scatter identifies the main cell population of interest.

Figure 1b



Following appropriate gating, the progenitor cells of interest are identified by their characteristic position to the left of the bulk cell population.

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PRODUCT	CODE
MoFlo High-Performance Cell Sorter	S2500

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Other vendor products used in this application: Sigma-Aldrich.

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.

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