MoFlo™ Viability and Function with High-Speed Sorting

Introduction

Sustained cell sorting at speeds in excess of 50,000 events per second is routine when using the MoFlo high-performance cell sorter. This powerful instrument gives academic and clinical researchers the capability to perform laboratory protocols that were previously unrealistic or impractical due to the length of sort time required when using other instrumentation at slower sort rates. In addition to purity and recovery, MoFlo utilizes cell viability and function as endpoint measurements to benchmark high-speed sort performance.

High-Speed Sorting Applications

High-speed sorting enables a wide range of novel applications, particularly those related to isolation of rare biological events.[1] Traditionally in this arena, scientists have relied on conventional flow cytometers in combination with technologies such as magnetic particle separation, centrifugal elution, density-gradient separation and complement-mediated lysis, to isolate specific cell populations. However, these technologies are time consuming and involve additional manipulation of the cell preparation that may lead to undesirable changes in cell function and/or cellular activation. High-speed sorting permits the use of a single purification step, delivering the cells of interest into culture or into the transfer recipient at >99% purity in the shortest possible time.

With the MoFlo, operators can define multiple cell populations, based on selectable parameters and simultaneously distribute those populations – even extremely rare ones – in up to four separate temperature-controlled receptacles. MoFlo's patented electronics process events at such high speeds that cells of interest can be retrieved from the original sample with a single pass through the flow cytometer, without prior enrichment steps. Interest in the use of high-speed sorting is growing for applications related to stem cells, dendritic cells, rare thymus-borne T cell precursors and genetic transfectants, which may make up as little as 0.01% of a given cell population. Operators can now routinely perform sorts on these populations in much less time than it takes to setup and run a magnetic column.

Instrument Design

The challenge of high-speed sorting is to find a way of rapidly processing events while maintaining the ability to detect even dim fluorescence with high sensitivity. By concentrating the cell sample and running at high pressures (typically 60 psi or greater), the diameter of the core stream is maintained, ensuring high fluorescent measurement precision. Furthermore, the pressure, combined with size of the nozzle tip, determines the frequency at which droplets may be stably formed. A higher droplet frequency correlates to increased yield, as the likelihood of having multiple cells within a single drop decreases. Therefore, fewer droplets are discarded due to event coincidence. While legacy-type sorters typically create 10,000-30,000 droplets per second, the MoFlo creates 90,000-200,000 droplets per second, significantly increasing the yield of every sort without sacrificing purity. Moving cells at these speeds requires careful design of the fluidics system, not only to maintain laminar flow, but also to ensure that sorted cells remain viable and fully functional after isolation.

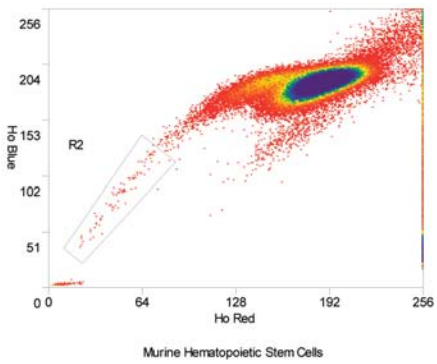

Figure 1

Murine Hematopoietic Stem Cells. Murine bone marrow is incubated with the nucleic acid dye Hoechst 33342, then analyzed using a MoFlo cell sorter. The Hoechst dye is excited using a 351 nm UV laser and fluorescent emission is detected both in the blue region, using a 450/20 bandpass filter, and in the red region, using a 675 eFLP filter. The progenitor cells of interest are identified by their characteristic position to the left of the bulk cell population. Acquisition and analysis were done using Summit software. Data courtesy of the University of Colorado Health Sciences Center.

Proven Performance

The MoFlo uses a patented nozzle design to reduce turbulence and minimize the effects of acceleration on each cell. This design has been extensively validated in peer-reviewed publications. Scientists worldwide routinely use the MoFlo to identify and segregate a wide variety of cell types including T cells, B cells, NK cells, dendritic cells, hematopoietic (Figure 1) and neural stem cells. Following sorting, these cells are fully functional and capable of cytokine production, antigen presentation, antibody production, activation, target-cell binding, post-transplantation engraftment and long-term culture.[2-32]

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