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Optimizing Cell Sorts

Introduction

There are several factors that need to be considered in order to prepare cells properly for a successful sort. Since there is a wide range of cell types used and experimental end-points, there is no single preparation methodology that is appropriate for all experiments. The following will attempt to elucidate some of the issues and allow for the researcher to determine what is required for their particular experiment.

Cell Size and Morphology

Researchers frequently sort cell types ranging from resting lymphocytes (small size, spheroid morphology, and robust) to aged microglia (large size, dendritic morphology and very fragile) and everything in between. The drop drive frequency (ddf) and stream pressure combinations are optimally matched to a particular nozzle size (50-130µm) depending on the cells being sorted. It is important for the researchers to be familiar with these issues relevant to the particular cell type being sorted.

There is a simple rule we follow that helps govern nozzle selection based on cell size:

The cell size should not exceed one-fifth of the nozzle diameter

This rule helps ensure that stream stability can be maintained during the sort. If larger particles are entering the stream, there is a deleterious effect on the droplet breakoff. A drifting breakoff can disrupt the careful calibration of the droplet delay and lead to problems ranging from fanning of the side streams (desired sample ends up missing the tube) to changing of the breakoff (severely compromises sample purity) to ultimately clogging the nozzle.

Morphology is a trickier subject due to the variation between cell types. In general, the more the morphology deviates from an ideal spheroid shape, the more susceptible the cells become to shear induced damage. We typically find that the greater the deviation from the ideal sphere, the larger the nozzle required by the instrument. It is important that you communicate information relative to cell morphology prior to scheduling a sorting experiment.

Sample Preparation

The single most important issue for a successful sort would have to be proper sample preparation. This can be broken down into four separate components:

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1. Single Cell Suspensions: In order for the sorter to function properly and to be able to deliver the proper results, the cells must be in a single cell suspension, and remain that way for the duration of the sort. This becomes a more important factor when working with adherent cell lines or tissues.
2. Optimized Sample Concentration: Cells must be at the proper concentration in order for the sorter to function optimally. Simply put, cells that are too concentrated will have a lower recovery due to coincidence aborts (two cell that are too close together will be rejected by the machine in order to ensure purity) and cells that are too dilute will have a longer processing time (or if they are processed faster, an increased signal CV).
3. Proper Sort Buffer Recipe: This is probably one of the most important factors to achieve an ideal sort. A properly designed buffer recipe will help maintain a single cell suspension as well as keep the cells in a good physiological state. Culture media is typically a poor sort buffer (although it can be modified). (see Sort Buffer PDF for more information)
4. Expedient Sample Processing: The sample must be prepared in as short a time as feasible to minimize stress on the cells as sorting is a relatively harsh process. Much of this can be achieved by simplifying the staining process and staggering the sample preps if more than one sample is being sorted.

Cell Physiology

Resting cells are typically very easy to sort, but most researchers have manipulated the system such that the cells are no longer in the most ideal state for processing. This can be addressed by setting up the instrumentation to run at lower pressures to minimize the stress on the cells. It is important for the researcher to convey any of these potential physiological issues to ensure the sort is properly configured.

End Point Requirements

The desired use for the sorted material can have a role in how the instrument is configured and how the sample can most efficiently be processed. Whether cells need to be viable, sterile or are used for DNA/RNA isolation can also have a role in instrument set-up.