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Application Note

Single Cell Isolation with Minimal Sample Input and High Cell Recovery

Introduction

Efficient isolation of single cells is a critical step for single-cell genomics, cell line development, and more. Traditionally, researchers have relied on cell sorters to isolate individual cells for these applications. However, aside from their pitfalls as high maintenance and operationally complex machines, traditional cell sorters also require cell samples to travel through long sample tubing, resulting in a high dead volume. To compensate for such loss, a large initial sample input is required. For precious cell populations, including primary or clinical samples, meeting this high sample input requirement can be impossible.

Namocell's single cell dispensers use disposable, microfluidic cell cartridges into which samples are directly loaded. As the sample flows through the cell cartridge, a proprietary mechanism sorts and dispenses cells within the cartridge in a single step, thus dramatically reducing the dead volume as compared to traditional cell sorters. Here, we evaluate the single cell isolation efficiency of Namocell's Single Cell Dispenser with a low sample input of only 100 cells with both adherent and non-adherent cell lines.

Methods

SP2/0 cells (non-adherent) and trypsinized HEK-293 cells (adherent) were counted and diluted to 5,000 cells/mL in culture medium. As calculated, every 1 µL contains ~5 cells. A total of 100 cells (20 µL) of each cell type was diluted into an additional 100 µL of PBS. For each cell line, these 100 cells were pipetted into a sterile cell cartridge (Namocell) and loaded into the Hana. Cell events were identified via the forward scatter (FSC) detection channel with a lower bound of FSC=50. Individual cells were dispensed into a 96-well plate with one cell per well. After the entire sample volume was dispensed, another 100 µL of PBS was added to the cell cartridge to wash out additional cells for maximal recovery.

Results

Cell recovery rates with and without washing are shown in FIGURE 1. Without washing, both cell types exhibited ~60% recovery. Subsequent washing resulted in a ~20% increase in recovery, for a final recovery rate of ~80%. There was no significant difference in recovery between HEK-239 and SP2/0 cells.

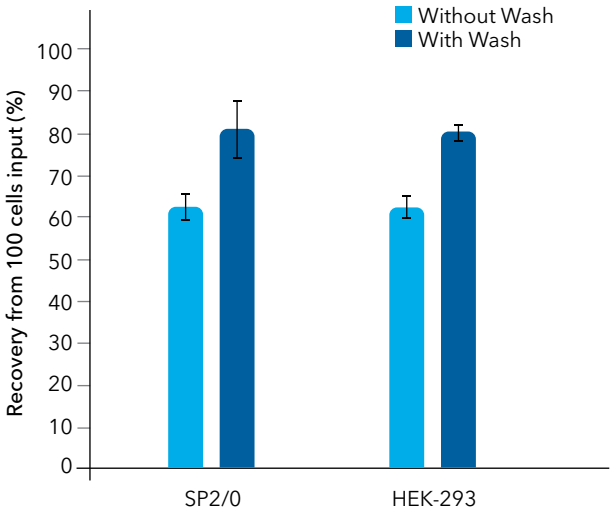

FIGURE 1. Single cell recovery rates from an initial input of 100 cells from SP2/0 (non-adherent) and HEK-293 (adherent) cell lines (n=5 replicates each). Error bars represent standard error.

Summary

Overall, the Namocell single cell dispenser can accommodate input sample with as few as 100 cells at a recovery rate around 80%, enabling efficient isolation of cells from extremely limited or precious samples.