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Application Note

Single Cell Deposition Efficiency

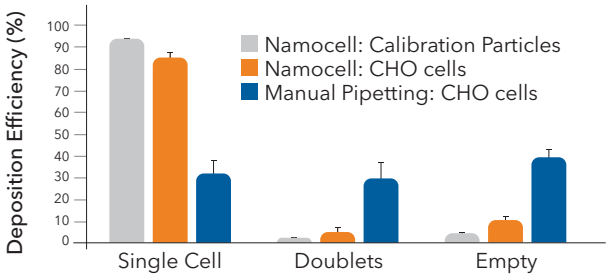
Introduction

Reliable deposition of single cells into tissue culture plates (one cell per well) is an essential consideration for clonal cell isolation and expansion. In a traditional cell line development workflow, manual limiting dilutions are used to generate single clones. However, the efficiency of cell deposition with this approach is inherently limited by the Poisson distribution, resulting in a waste of both consumables and time. Namocell's single cell dispensers provide a fast and reliable alternative for single cell isolation, alleviating the need for manual pipetting while preserving cell integrity by sorting at a gentle pressure of <2 psi. In this application note, Namocell's dispensing efficiency is first established via deposition of fluorescent calibration particles (beads). Next, deposition of single CHO cells is compared between Namocell's dispenser and manual limiting dilutions. The efficiencies reported here serve as a reference for the expected deposition efficiency with Namocell's platform regardless of downstream application (cell line development, single cell genomics, etc.), though results will vary across different cell types and cell preparation approaches

Methods

First, fluorescent calibration particles (Spherotech) were dispensed into 96-well plates using the single cell dispensing mode of the Namocell Single Cell Dispenser. Next, semiadherent CHO cells were resuspended in culture medium and filtered with a 35 µm cell strainer (E&K Scientific) to remove clumps of cells. The resulting single cell suspension was inspected under a light microscope to verify a high-quality single cell suspension (devoid of clumps and doublets). The cell suspension was then stained with the cell-permeant viability dye Calcein AM (Thermo Fisher Scientific) and the stained cells were subsequently dispensed into 96-well plates similarly as the calibration particles. For both calibration particles and labeled CHO cells, the FITC detection channel (533 nm) was used as a trigger to identify cell events. As a control, the same CHO cells were dispensed into 96-well plates using manual limiting dilutions, with a target of one cell per well. For each condition, seven plates were collected over the course of seven days, among three independent Namocell instruments. Visual inspection under a light microscope was used to score each well of the 96-well plates. Deposition rates were calculated for single cells, doublets, and empty wells.

Results



The deposition efficiencies of each experimental condition are reported in FIGURE 1 (above). With calibration particles, Namocell's single cell deposition efficiency was on average 93%, with 2% doublets and 5% empty wells. For live CHO cells, efficiency of single cell deposition was on average 85%, with 5% doublets and 10% empty wells. For the plates dispensed via manual pipetting, efficiencies were 32% single cells, 29% doublets, and 39% empty wells. Across all plates of CHO cells dispensed using the Namocell instruments, the highest rate of single cell deposition observed was 92%. For manual limiting dilutions, variability across replicates was the highest of the three conditions (FIGURE 1).

Summary

Overall, Namocell's single cell dispensers yield a dramatic improvement in deposition efficiency over limiting dilutions, while simultaneously providing the ability to sort multiple plates in a matter of minutes, thus saving time, energy and consumables.