

Sorting Single-Cell Suspension CHO Cells Optimized for High Outgrowth

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Introduction

Suspension cell lines are critical for the scaled-up production of proteins and biologics intended for research and clinical use, respectively. Suspension cells producing biologic drugs need to be grown serum-free to avoid using inconsistent animal derived serums, having to use purification methods to eliminate serum proteins during downstream processing, and to avoid the potential of introducing harmful microbes, viruses or prions.^{1,2}

In addition, cell line development and single-cell cloning have previously depended on inefficient methods such as limiting dilution or complex high-pressure cell sorters that negatively affect cell outgrowth.^{3,4} Moreover, low monoclonality rates can lead to genetic heterogeneity when producing cell banks or for antibody production.⁵

With the WOLF Cell Sorter and the N1 Single-Cell Dispenser, users can rely on an easy-to-use platform for suspension cell outgrowth that relies on serum-free preparation while maintaining high viability and monoclonality.

Methods

Suspension CHO-ES cells (Expression Systems, catalog # 94-008F) were expanded and maintained in serum-free ESF SFM Mammalian Cell culture medium (Expression Systems, catalog # 98-001). Cells were prepared for single-cell sorting using the WOLF by diluting the cells in sample buffer composed of ESF SFM medium + 25 mM HEPES (Gibco, catalog #15630080). The sheath buffer was also composed of ESF SFM medium + 25 mM HEPES. Cells were prepared to have a final sorting concentration of 1×10^5 cells/mL. Suspension CHO-ES cells were sorted based on their scatter properties using a singlets gate without the use of viability dyes (i.e. label free).

The cells were sorted using the N1 Single Cell Dispenser into a total of three 96-well plates that were pre-filled with 200 μ L of optimized cloning medium. Optimized cloning medium was composed of 80% EX-CELL CHO cloning medium (Sigma, catalog # C6366) that was optimized with 6 mM GlutaMAX (Gibco, catalog # 35050061), 20% ESF SFM medium, 1X

MEM Non-Essential Amino Acids (Gibco, catalog #11140050), and 1:80 ClonaCell-CHO ACF supplement (STEMCELL, catalog #03820). The testing conditions were repeated to complete a total of three WOLF Cell Sorter cartridges and nine 96-well plates.

In addition, one WOLF Cell Sorter cartridge was used to perform the same sort, but cells were sorted into three 96-well plates that were pre-filled with 200 μ L ESF SFM Mammalian Cell culture medium as a negative control.

For all sorted plates, well D4 was spiked with the stock CHO-ES cells to aid in focusing when imaging the plates. The plates were stored in the incubator at 37°C, 5% CO₂ for 14 days. On Day 7 and Day 14, the plates were imaged using the Celigo (Nexcelom) with only the brightfield channel to confirm monoclonality and outgrowth.

Suspension CHO-ES Monoclonal Outgrowth

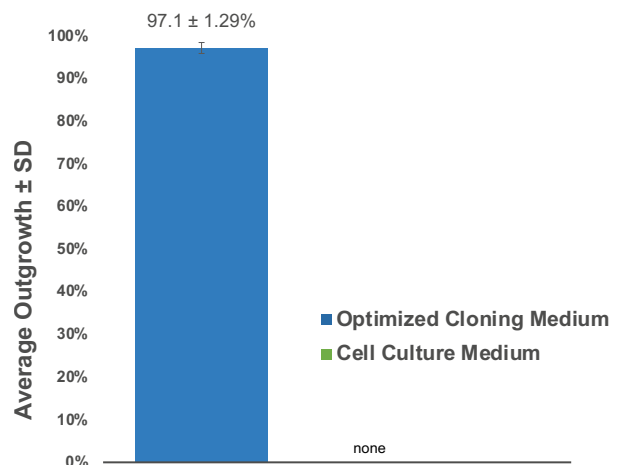


Figure 1. Comparison of Single Cell Outgrowth based on Type of Cloning Medium Used: Average monoclonality when using the WOLF and sorting into optimized cloning medium was of $97.1 \pm 1.29\%$ ($n = 9$). Meanwhile, when sorting into 96-well plates with regular cell culture medium no outgrowth was seen ($n = 3$). Optimized medium and the WOLF ensured high outgrowth with high monoclonality.

Results

Using optimized cloning medium and the WOLF we observed an enhanced outgrowth of the suspension CHO-ES cell line. The average monoclonal outgrowth analyzed on Day 14 was of $97.1 \pm 1.29\%$ when sorting into optimized medium compared to no outgrowth when sorting into regular cell medium (Figure 1).

High outgrowth and efficient monoclonality can be observed when visualizing plates sorted with the WOLF into optimized cloning medium (Figure 2A). The WOLF resulted in an average total outgrowth of $93.4 \pm 1.13\%$ with $91.0 \pm 1.01\%$ of the wells containing a single colony with only one round of single-cell cloning (Figure 2B).

Other groups have reported limiting dilution for suspension CHO cells cloning with outgrowth efficiencies of less than $38.3 \pm 10.4\%$ after optimization.⁶ This further demonstrates that limiting dilution has low efficiency in producing monoclonality that is less than the predicted Poisson distribution.⁷ Furthermore, highly inefficient limiting dilution requires more cloning rounds to ensure monoclonal cell clusters.⁸

Conclusion

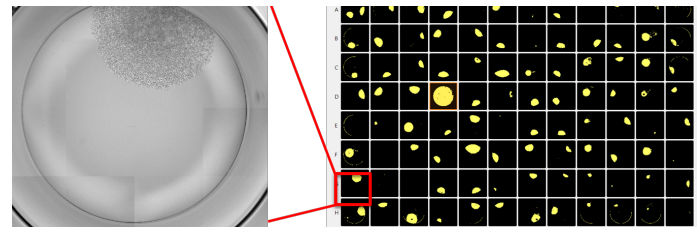
The WOLF Cell Sorter is a critical tool for cell line development workflows that can be used for label-free or fluorescent activated single-cell sorting of suspension cell lines growing in serum free media. Additionally, the WOLF is capable of dispensing a single cell per well gently and with high accuracy to ensure monoclonality needed for cell line development and antibody production.

For more information, visit nanocellect.com or email info@nanocellect.com

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(A)



(B)

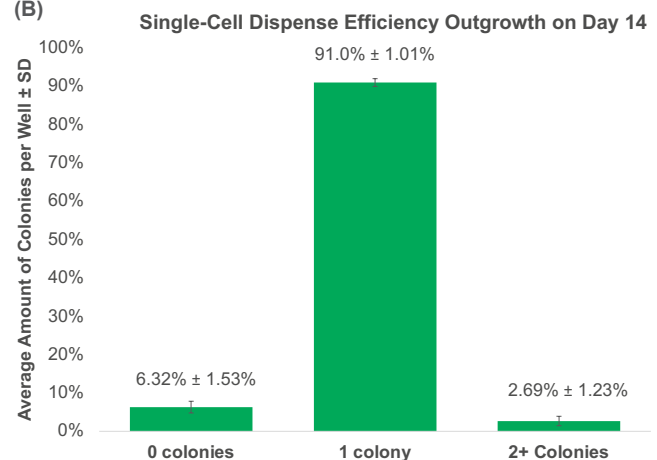


Figure 2. The WOLF Ensures High Monoclonality and Outgrowth: (A) A representative 96-well plate sorted with the WOLF onto optimized cloning medium imaged on Day 14 showed a high number of wells containing a single colony that originated from a single cell. Well D4 was spiked to facilitate plate imaging. Average total outgrowth when using the WOLF was of $93.4 \pm 1.13\%$ (B) The WOLF demonstrated an average single-cell dispense efficiency of $91.0 \pm 1.01\%$ with $6.32 \pm 1.53\%$ of the wells having no colonies and $2.69 \pm 1.23\%$ had two or more colonies per well.