Application Note: cellenONE® Unparalleled clonal recovery achieved in 96 and 1536 Microtiter Plates

Abstract

The main challenges associated with cell line development are maintaining high cell viability and ensuring monoclonality of isolated cells. Unlike flow cell sorters, which induce high stress and compromise cell viabilities, automated cloning using cellenONE is a gentle and precise method to achieve unparalleled clonal recoveries in both standard (96 MTP) and high density (1536 MTP) substrates. This is demonstrated with CHO cells, one of the most common cell line used in recombinant therapeutic proteins manufacturing.

Materials and method

CHO cells were passaged two days before isolation and cultured under standard conditions (DMEM/F12 with 10% FBS and Penicillin, Streptomycin, Amphotericin-B at 37˚C in 5% CO2).

Prior to cloning, cells were washed twice with PBS, detached from their culture plates (0.5 mL trypsin for 1 min at 37ºC), centrifuged (250g for 5 min at 4ºC) and resuspended in PBS (200 cells/µL). The cell suspension was stored on ice and diluted to 100 cells/µL in degassed PBS immediately before cloning.

Target plates were prefilled with media (200 µL/well for 96 Microtiter Plates (MTP); 12 µL/well for 1536 MTP), equilibrated at 4˚C then transferred onto cellenONE's target holder pre-cooled to 2ºC.

Using cellenONE X1, single cell isolation (Figure 1) was completed in approximately 4 min and 46 min for 96 and 1536 MTP, respectively. The plates were then cultured under standard conditions for 14 and 7 days for 96 and 1536 MTP (Figure 3), respectively.

Plates were imaged using an automated microscope (Zeiss Observer Z1) and each well was inspected for the presence of single, multiple or no colonies.

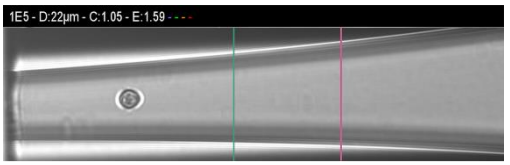

Figure 1. Image of an isolated CHO clone in cellenONE's dispenser, with corresponding cell measurements (diameter, circularity, elongation) and culture well location.

Results and Discussions

The number of wells containing single CHO colonies were counted for each plate (six 96 MTP and one 1536 MTP) and the percent of wells containing single colonies was calculated (Figure 3).

Outstanding clonal recoveries were observed with 96% and 94% of wells containing single colonies in 96 and 1536 MTP, respectively (Figure 2). Additionally, the remaining wells were empty and did not contain multiple colonies. To our knowledge, this is the first time such high clonal recoveries have been demonstrated with CHO cells in both standard (96 MTP) and very high density (1536 MTP) substrates.

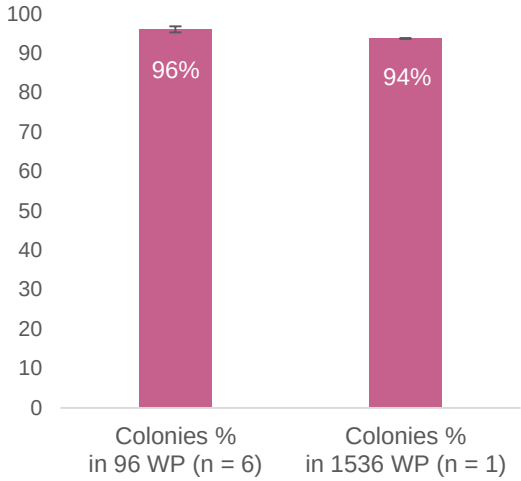

Figure 2. Clonal recovery in 96 and 1536 MTP after 14 and 7 days of culture, respectively.

Conclusions

This experiment demonstrates the cellenONE as an ideal platform for undertaking automated cloning in both standard and high density substrates as it ensures monoclonality while maintaining outstanding clonal recovery.

Compatibility with high density substrates offers a dramatic increase in throughput to automatically process the equivalent of 16 individual 96 MTP in under an hour leaving personnel free to perform less routine tasks.

In addition to saving time, one can save on consumables and expensive reagents while ensuring documentation and monoclonality of all isolated cells.

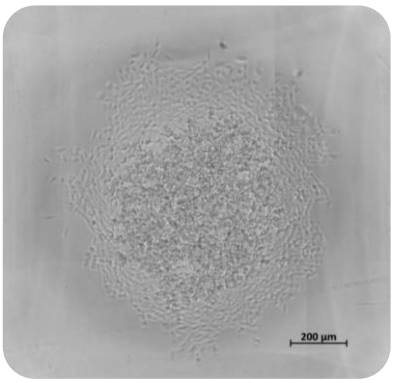

Figure 3. Example of a colony after 7 days of culture in a well of a 1536 MTP.

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