BECKMAN COULTER
Life Sciences

Considerations of Cell Counting Analysis when using Different Types of Cells

The Vi-CELL BLU analyzer leverages the key performance features of the Vi-CELL XR analyzer but incorporates many design improvements that our customers have requested over the years.

While a seemingly straightforward application, automated cell counting can be influenced by a variety of conditions and variables arising from both the sample and instrument. With the Vi-CELL BLU analyzer we have introduced the capability to load 96 samples into a standard plate and run this as a single experiment. However this requires approximately 3 hours to run the entire plate which may not be ideal, particularly for cell types that generally have low viability or are prone to deterioration once outside the incubator or bioreactor.

The plate loader also allows us to assess multiple different experimental conditions in a single run thus providing the opportunity to evaluate a range of conditions or criteria.

To demonstrate these capabilities we reviewed several different cell types and cell preparation approaches using standard cells.

Figure 1. Vi-CELL BLU Cell Viability Analyzer.

Cell Counting Analysis

Cell cultures of CHO (Chinese Hamster Ovary), EL4 (Mouse T Lymphocyte Cell Line), SF9 (ovarian tissue from ***Spodoptera frugiperda***) insect cells and of HELA cells were used to assess the effects of long analysis durations and different sample preparation conditions on different types of cells.

CHO Cell Sample Preparation Impact

Centrifugation and resuspension are routine methods for washing and concentrating cells ahead of reseeding or use in other experiments. CHO cells are generally considered to be quite durable, but they do have very particular growth requirements and require specialized media for best health. In the experiment below the cells were centrifuged at 1000 x g for 5 minutes, a relatively mild spin before being resuspended in media or buffer to assess the impact, if any, of cell preparation on cell health.

Results

Treatment	Concentration (M/mL)	Viability (%)	Diameter (µm)
Untreated	4.83	94.30	16.12
1 Spin, resuspend in media	5.05	94.48	16.17
1 Spin resuspend in PBS	4.70	65.69	14.17
2 Spin resuspend in PBS	4.45	53.77	13.99

The results show that centrifugation and resuspension in recommended media has no appreciable impact on the CHO cells. However, resuspension in PBS buffer results in a drastic loss of viability and significant reduction in average cell diameter. There is also some minor reduction in cell concentration which may be expected from losses during resuspension. Spinning and resuspending a second time in PBS has a further negative impact on cell viability and cell size.

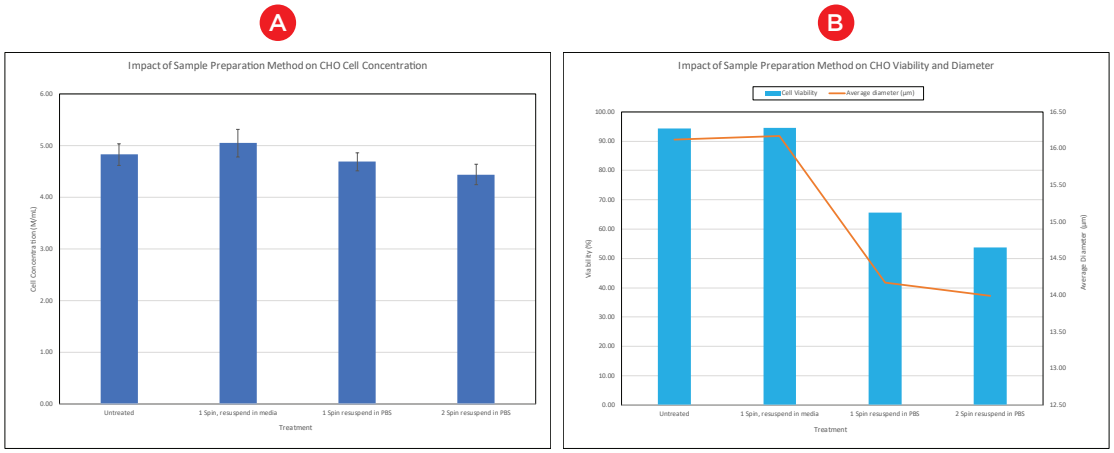

Figure 2. Impact of sample preparation method on CHO cells.

EL4 Cells Mixing Cycle Analysis

EL4 cells are generally considered to be fragile and even under ideal conditions grow slowly and show viability <70%. EL4 cells were prepared and dispensed into a 96-well plate. Cell type profiles were created using the standard mammalian cell profile as source and differing levels of mixing cycles. The goal was to assess the impact, if any, of increased agitation on a cell type known to be easily damaged by vortex mixing and centrifugation.

Results

The results for the EL4 cells are given below.

Mix Cycles	Cell Count	Viable Cell Count	Concentration (M/mL)	Viability (%)	Diameter (µm)
1	4648	2837	1.72	61.06	11.34
3	4637	2715	1.72	58.54	11.34
5	4660	2582	1.73	55.69	11.34
7	4660	2448	1.73	52.53	11.32

While the cell counts, concentration and diameter values show no statistical significance there is a marked drop in viable cell count and consequently, in cell viability with increased mixing cycles across all dilutions. This may indicate that a cell type profile with reduced aspiration and mixing cycles may be more suitable for this type of cell, and excessive agitation may not be ideal and should be avoided.

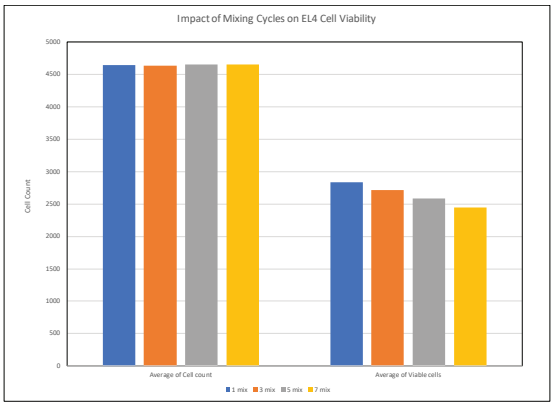

Figure 3. Impact of mixing cycles on EL4 cells.

SF9 Insect Cells

Even when grown under ideal conditions SF9 cells do not achieve high densities easily, and typically have viability below 60%. It is therefore possible that the prolonged instrument time required for a plate run may negatively impact the cell culture. To assess this, SF9 cells were plated and run using the standard Insect Cell profile.

The results show that over the course of the 3-hour run there was no detectable impact on cell viability and no notable change in cell diameter that may occur if the cells are dying or swelling. As such it would appear this cell line, although low in viability, is robust enough to spend extended periods outside the cell incubator environment without adverse effects.

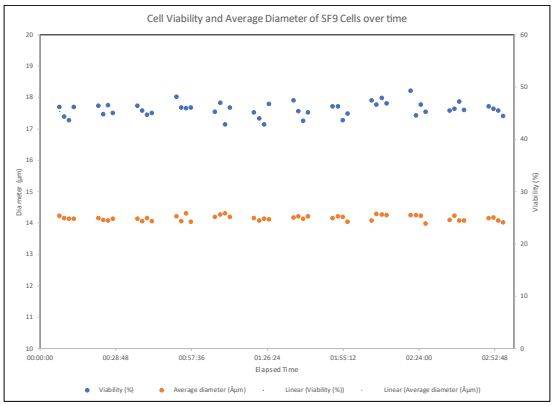

Figure 4. Viability and Average Diameter of SF9 cells overtime; no significant change noted over course of experiment.

HELA Cell Cultures

HELA cells were grown to a high state of confluence in order to stress the cells. The cells were then harvested and dispersed into suspension. The cells were then plated out on a 96-well plate and data collected using standard mammalian cell profile. The plate was run collecting data by columns to ensure the same time interval between samples in the rows.

It was noted that the later samples in the plate run were reporting slightly higher concentrations. Further investigations showed that the cells were increasing in average diameter as time progressed.

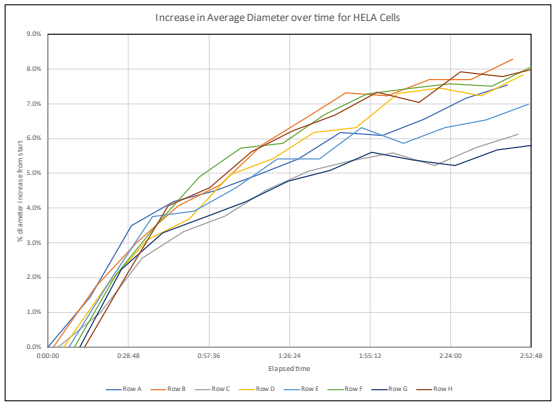

Figure 5. Percentage increase in average diameter of HELA cells over time.

Viewing the acquired images shows an increasing number of cells which appear to be swelling or blebbing along with a large number of non-cellular vesicles. While the latter do not usually impact the cell count, depending on the cell profile settings, they could be counted as small cells. The swelling effect causes the average cell diameter to increase almost 10% over the course of the 3 hours it takes to run the plate and may cause some smaller cells just below the lower diameter threshold to become included in the cell counts.

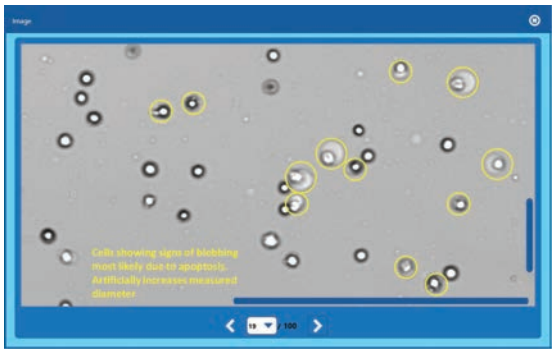

Figure 6. Cell swelling and blebbing in HELA cells after >2hrs outside incubator.

Conclusions

It is recommended that prior to running a cell culture on a complete plate that some initial evaluation is performed to determine the robustness of the cells involved and their ability to tolerate the sample preparation methods and prolonged exposure outside the incubator environment. Even engineered cells such as CHO cells or immortal HELA cells, which are generally selected to be quite durable, can be effect by stress or particular sample preparation conditions.

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