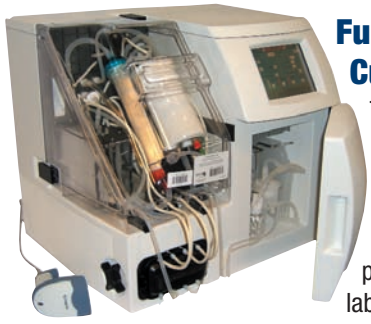
EMERGING TECHNOLOGIES IN

Advantage Business Media

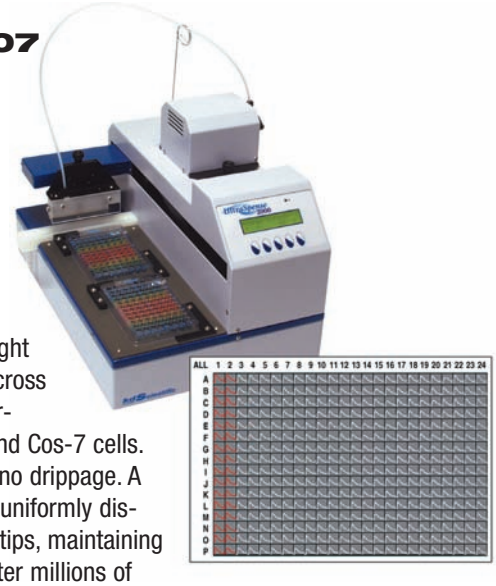
Life Science

Tools for Discovery ■ www.BioscienceTechnology.com ■ March 2007



Fully Automated, Reusable Cell Culture Instrument

The AutovaxID automated, reusable cell culture device uses a fully disposable, closed-system cell-growth chamber that incorporates a hollow-fiber cell-growth cartridge. This bioreactor replaces conventional stainless steel, glass and plastic cell-growth chambers that require more laboratory space. Because it is fully enclosed and automated, it requires almost no supervision. The device enables scalable production of cells or cell-derived products, including monoclonal antibodies for personalized medicine applications. It is suitable for growing antibody-secreting cell lines, including hybridomas and Chinese hamster ovary (CHO) cells. The system operates with both suspension- and attachment-dependent cells. Potential applications include growth and expansion of an array of cell types, including patient-specific cells for emerging cell-based and cell-derived therapies. The device enables production of personalized cell-based treatments in compliance with U.S. Food and Drug Administration Good Manufacturing Practices (GMPs). **Biovest International, Inc.** www.biovest.com, 508-793-0001



Dispensers Feature Specially Designed Manifold

UltraSpense 2000 dispensers deliver a uniform distribution of live cells into 384-well microplates with tight seeding density variability across the plate. The assay was performed with CHO, Hek293 and Cos-7 cells. There is no edge effect and no drippage. A specially designed manifold uniformly distributes material to all eight tips, maintaining a consistent performance after millions of dispensing cycles. This is combined with a proprietary algorithm to handle the pumping that overcomes the natural tendency for fluids to accumulate on the outside of the manifold tips. The pump has just one moving part, delivering accuracy and consistent volumes across a range of viscosities. **KD Scientific, Inc.** www.kdscientific.com, 508-429-6809

Adapting Automated Cell-Based Assay Washing Procedures

■ *by Ted Quigley*

Figure 1. *The ELx405 Select CW automated microplate washer from BioTek Instruments.*

Since the inception of cell-based assays in microplate format, washing steps have been among the most common sources of error. In developing cell-based assays, investigators begin with manual microplate washing methods using squeeze bottles or multi-channel pipettes. As the number of samples and microplates increase, developers begin to automate the assay. In this process, the conversion of cellular assays to automated washing is often the most difficult. Automated microplate washers (Figure. 1) can remove cell layers and increase coefficients of variation to unacceptable levels. By their nature, manual wash methods incorporate soaking, which is forgotten or overlooked when converting a washing procedure to an automated microplate washer.

In manual cell-based assays, using a multichannel pipette is the most common method for washing microplates. Manual pipetting enables control over the force of the fluid direction as it is added as well as its direction toward the sides of the wells to avoid the cell layers. Aspiration uses the "flick & tap" method, which involves inverting the microplate rapidly over a sink or receptacle, and tapping the plate over absorbent paper to remove any remaining liquid. It's quick, but repeated tapping of many microplates on absorbent towels can lead to repetitive stress injuries. In addition, there is always the possibility of cross-well contamination, cell damage, inconsistent technique, and even potentially

Continued on page 42

Continued from page 40

harmful aerosols. If using the pipette to aspirate the fluid layers above the cells, there is usually residual liquid remaining in the wells, and the amount may vary throughout the microplate, leading to inconsistent results. Additionally, special care must be taken to ensure that the pipette tip does not disturb or damage the fragile cell monolayer.

A logical choice would be to move to an automated microplate washer, especially when looking for increased throughput consistency or safety within the process. Automated microplate washers significantly reduce human error, require little to no technical experience, and produce extremely consistent results through every well and every microplate. The automated process is not necessarily "plug-and-play", however, and several parameters deserve close inspection to ensure that the automated washing process is optimal for the cell-based assay being run. If an existing microplate assay is converted to an automated washing method, the characteristics of the manual washing method mentioned above should be considered when developing the new automated process.

Every conversion of cell-based assays from manual to automated washing methods should be customized to the needs of your particular laboratory. Some parameters should remain constant, while others may be modified. For example, the number of automated wash cycles should always equal the number of wash cycles in the manual method. Additionally, the dispense volume should fill the wells as much as possible in each wash step to ensure adequate dilution of the fluid layer above the cells. The dispense height is also unlikely to require adjustments in this development process. Finally, the time of aspiration at the stop position just above the cells should be as short as possible.

At a glance

- Washing steps are a common source of error in cell-based assays in microplate format
- If using a pipette to aspirate fluid layers above cells, liquid remaining in wells can lead to inconsistent results
- If an existing microplate assay is converted to an automated washing method, the characteristics of the manual washing method should be considered
- Conversion of cell-based assays from manual to automated washing methods should be customized to the needs of each lab

There are, however, a number of variables that should be adjusted to suit your individual cell-based assay processes:

Fluid Flow Rate.

Excessive flow rate can dislodge cells even if a substantial residual remains to protect the cells. Some cellular assays require a stream of drips to be dispensed into the well. Other cellular assays can tolerate a slightly faster continuous stream.

Horizontal dispense position.

The direction and force of wash buffer flow can disturb the cell layer. With the flow rate fixed at the lowest possible rate suitable for cell-based assays, the horizontal dispense position should direct the fluid flow as far to the side of the well as possible to gently wash the cell layer with minimal disturbance.

Aspiration height.

When aspirating with an automated microplate washer, a residual buffer layer of 20 to 100 mL is necessary to protect the fragile cell layers from dispensing and aspiration forces. A low aspiration height may in fact remove cells from the plate, and on the other hand, a high aspiration height may leave too much buffer in the wells. It is recommended that the aspiration height be initially positioned in the middle range of the automated microplate washer, and adjusted accordingly to find the height that best suits the application.

Aspiration rate.

The aspiration tube will exert some vacuum force, creating a vortex that may disturb or damage cell layers. The aspiration rate should be set to a cell wash aspiration rate, which features a movement profile different from those required for ELISA assays. To start, choose the slowest possible cell washing rate to minimize the vortex created by the aspiration tube. Faster rates can be chosen in comparison if cells are still being removed.

Aspiration force.

If necessary, aspiration forces may be reduced. As vacuum force decreases, buffer aspiration may be reduced; this is especially true in 384-well microplates. Therefore, if a reduction in vacuum force occurs, the residual wash buffer level should be checked carefully, and readjusted as necessary.

> "Careful attention to detail when converting from manual methods to automated methods via microplate washers will reduce time and operator error normally associated with manual cell-based microplate assays."

Soak time.

If the variability between assay replicates is high and the above variables have been optimized, then incorporating a soaking period of wash buffer with the sample may lessen the observed variation. Generally, longer soak times do not harm assay results, but more than three minutes has not been shown to improve results. One minute may not be enough to show the maximum benefit of a soaking phase for certain assays. It is recommended to start with three minutes, and decrease the previous soak times by half to determine the minimum soak time that would be sufficient.

Once all changes have been applied, it is always good practice to review the entire process, fine-tuning and safeguarding against any anomalies. An initial investment of time and careful attention to detail when converting from manual methods to automated methods via microplate washers will, in the long run, significantly reduce time and operator error normally associated with manual cell-based microplate assays. In addition, automated microplate washers provide increased repeatability and throughput, both critical factors in cell-based assay applications. ■

Ted Quigley, Applications Specialist, BioTek Instruments, Inc., may be contacted at quigleyt@biotek.com or by phone at 888-451-5171 x222.

Online

For additional information on the company discussed in this article, see Laboratory Equipment magazine online at **www.LaboratoryEquipment.com** or the following Web site:

■ **www.biotek.com**