Poster Presentation

Using the ELx405™ Select CW Microplate Washer to Wash Loosely Adherent Tissue Culture Cells

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Abstract

The use of cell cultures in large numbers of samples to test agents has become commonplace in today's HTS/HCS environments. Screening assays that utilize cells grown in culture often requires one or more wash steps to remove unwanted substances or solutions. For example, prior to microscopic examination of fluorescently stained cells, the media needs to be replaced with an inert buffer such as PBS. After fixation, excess formaldehyde is removed prior to antibody staining, after which the unbound labeled antibody is removed by a washing step. These steps require the addition and removal of buffer solution without disruption of the monolayer of cells present in the wells of the microplate. While the many cultured cells do not present a problem when they are washed, loosely adherent cells often do. Loosely adherent cells do not attach to the microplate substrate strongly and in many instances will form loose aggregates or piles rather than a uniform monolayer. As a result, they are often dislodged when fluid is dispensed at too high a pressure by the plate washer, resulting in their loss with subsequent aspiration of the fluid present in the well. To address these problems with washing loosely adherent cells, BioTek Instruments has developed the ELx405 Select™ CW microplate washer. Here we describe the use of the ELx405 Select CW microplate washer to wash the loosely adherent HEK293T cells with PBS in both 96- and 384-well microplates and compare its performance to a standard microplate washer.

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Figure1. ELx405™ Select CW Microplate Washer

Introduction

The ELx405™ Select CW builds on the proven design of the standard ELx405™ Select (Figure 1). It incorporates BioTek Instruments' patented manifold design to overcome the difficulties presented by high-density microplates and allows virtually the same functionality in 16x24 matrix 384-well plates as expected with the 8x12 formatted 96-well microplate. Dispense and aspiration manifolds are physically separated and arranged on top of each other. The lower manifold (dispense) is constructed in such a manner as to allow the tube from the upper manifold (aspiration) to pass through and enter the well of the microplate. In order for the dispense pipe to be able to dispense fluid into a small well while the aspirate pipe is removing fluid from the same well, as is the situation when overflow and bottom washing is performed, the dispense pipe is angled. This allows for the dispense pipe to be offset from the center of the well, providing room for the aspiration pipe, yet still allowing the fluid jet to enter the well from the side. This canted design also has the added benefit of imparting a gentle swirling motion to the fluid that results in a more effective wash. In addition, the ELx405 Select CW incorporates a dual fluid path and software control designed specifically to lower the dispense rate to the lowest possible flow, without affecting dispense accuracy and precision (Figure 2). When the new low-flow rates are selected from the keypad menu, the flow control valve directs all fluid movement through the "Low Flow Line", which has the ability to restrict flow to very low rates. When standard rates are selected, the flow control valve opens, allowing full fluid movement through the system.

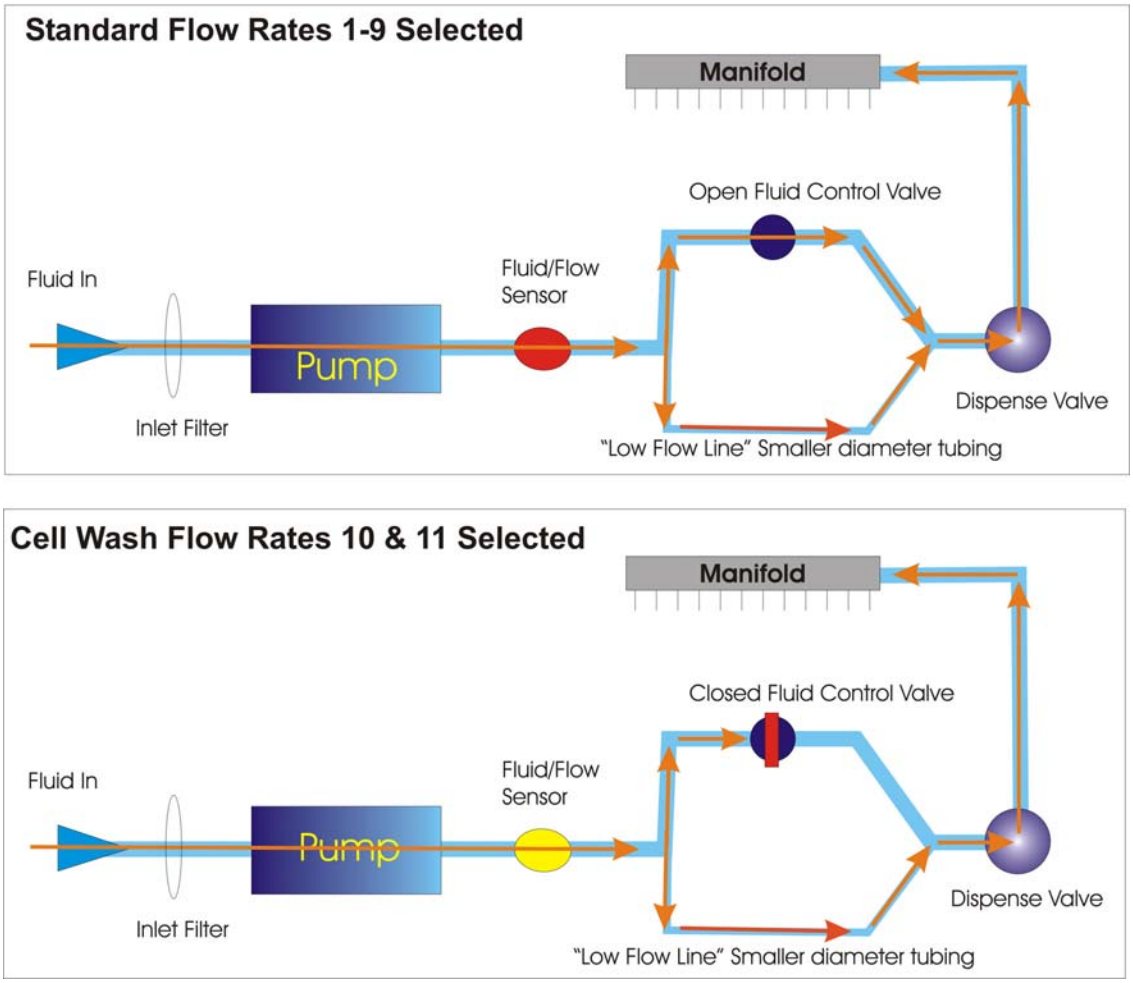

Figure 2. Bifurcated fluid path of the ELx405™ Select CW. When using standard dispense-rates (rates 1-9), both pathways of the bifurcated fluid path are open. Fluid enters through the inlet filter and dispense volume is controlled by the dispense valve. If low flow rates (rates 10 and 11) are selected, the fluid control valve closes blocking the larger main fluid line. Fluid flow is now restricted to the narrower "low flow line" allowing for much lower fluid flow rates, while maintaining dispense accuracy.

Materials and Methods

HEK293T cells were cultivated in DMEM (10% FCS) and plated into Corning® Costar 96- or 384-well plates (P/N 3614 and 3712 respectively) at 37°C. The following day, randomly selected wells were photographed using a Discovery-1 Screening System (Universal Imaging Corp) with 2x and 4x light transmission objectives. After taking the baseline image, plates were then washed with PBS (3 wash cycles) using an ELx405 Select CW programmed with a dispense rate of 11, which is optimal for loosely adherent HEK293T cells. After washing, the same wells were re-photographed to ascertain the utility of the cell wash programming. After determination that the cells were unchanged using the optimized washing protocol, the plates were re-washed using rate 1 (which had previously been found to work with strongly adherent cells), and photographed a third time. All photographic data was recorded using an integrated CCD camera saved as digital files that were collated using Adobe® Photoshop®.

Results

Figure 3 illustrates the mechanism by which loosely adherent cells could be disrupted from the bottom of microplate wells by the dispense fluid. When an excessive fluid rate is used to wash loosely adherent cell lines, such as human embryonic kidney (HEK293T) cells, a significant portion of the cells are disturbed. As demonstrated in Figure 4, when 293T cells in a 96-well plate are washed with a rate setting of 1 (204 µl/tube/sec) using the ELx405 Select CW, large portions of the well surface are observed to be denuded of cells (Figures 4B and 4D) after washing. This area generally was located to the left side of the well, which corresponds to the side toward which the PBS buffer fluid was dispensed. The same phenomenon is observed when 384-well plates are washed. When dispense rate 1 (204 µl/tube/sec) is used in the wash cycle, a significant number of cells are lost in a pattern very similar to that observed with 96-well plates (Figures 5B and 5D). Higher magnification (4x objective) further illustrates the loss of cells from the bottom of the microplate well (Figure 5D).

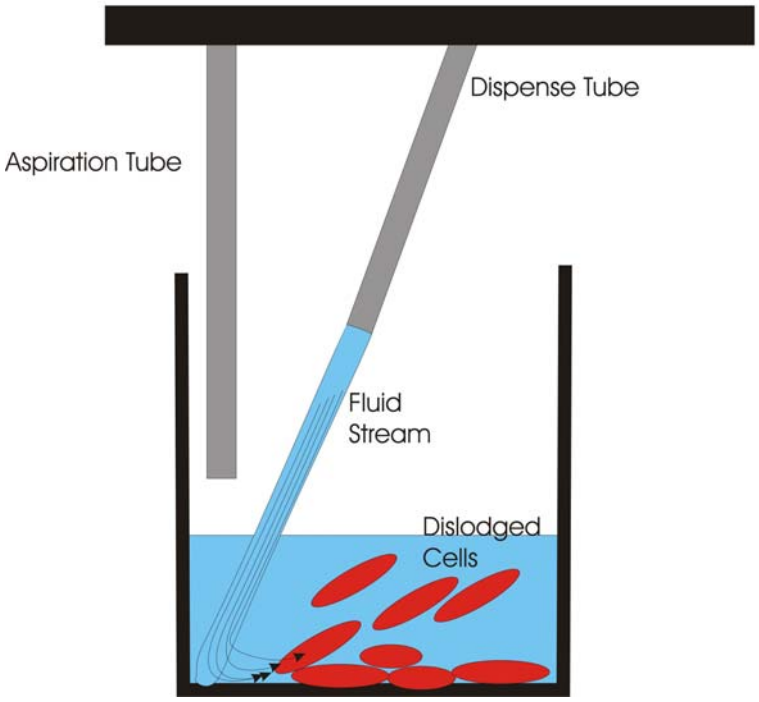

Figure 3. Illustration Depicting Cell Dislodgement. Fluid dispensed at too high a flow rate will dislodge loosely adherent cells into the well fluids. The dislodged cells will susequently be removed during aspiration.

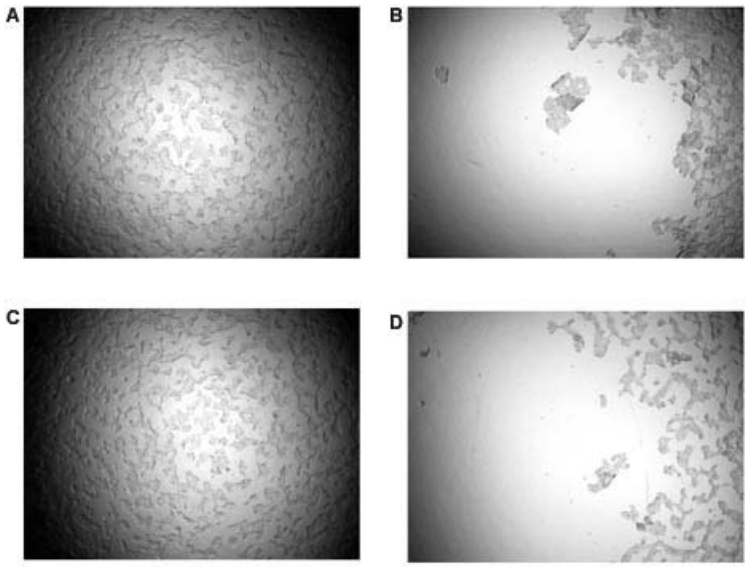

Figure 4. Before and after washing 96-well plate with cells using standard dispense rate. Two different wells of a 96-well plate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2x objective.

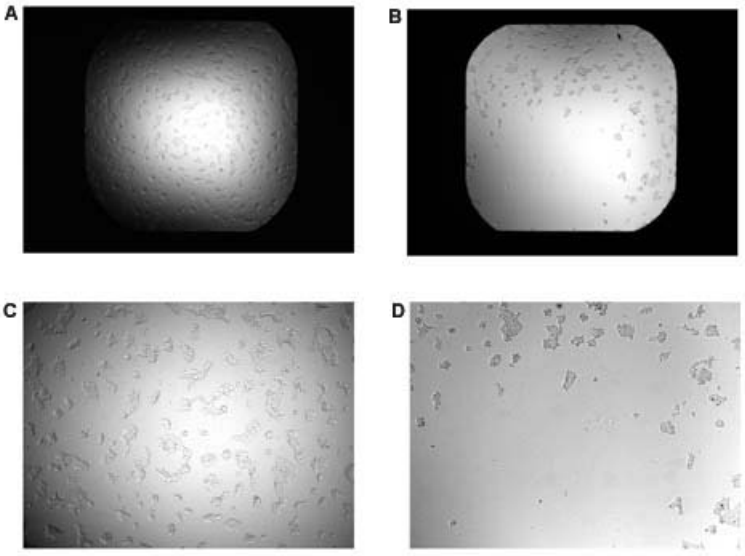

Figure 5. Before and after washing 384-well plate with cells using standard dispense rate. Two different wells of a 384-well microplate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. Note that the top two images (A and B) were obtained using the 2x objective, while the bottom two images (C and D) were obtained using the 4x objective.

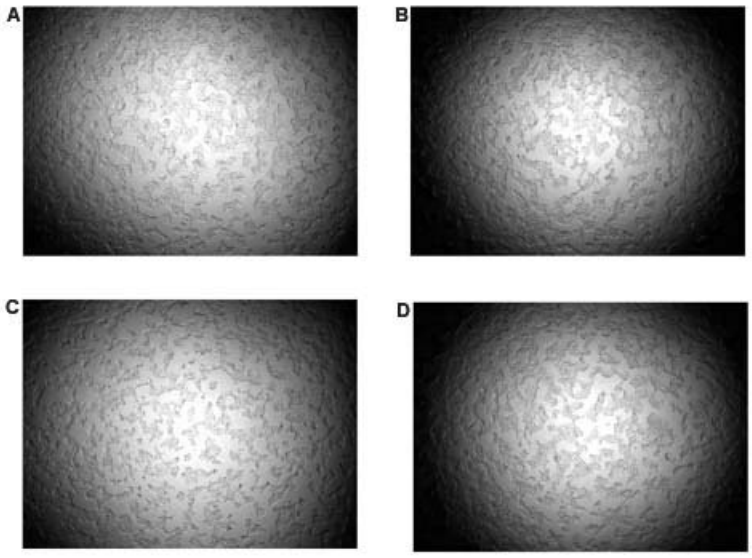

Figure 6. Before and after washing cells using low flow rate setting. Two different wells of a 96-well plate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2x objective.

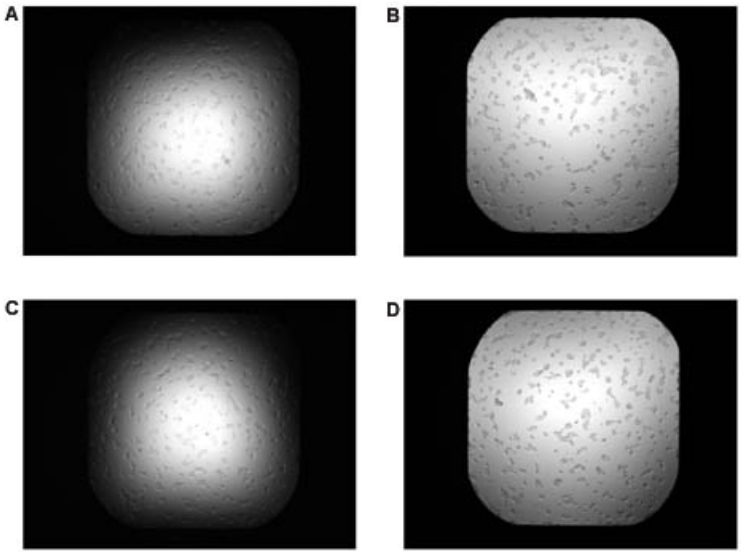

Figure 7. Before and after washing 384-well plate with cells using low flow rate. Two different wells of a 384-well microplate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2x objective.

When the low flow rate 11 (116 µl/tube/sec) is enabled, there is virtually no change to cell number or morphology when washing HEK293T cells. When the images from two different wells obtained before (Figure 6A and 6C) and after (Figure 6B and 6D) washing with PBS for 3 cycles are compared, virtually no change is apparent. The cells remain attached and viable for further experimentation. Note that these two wells were found to be representative of the entire 96-well microplate. Figure 7 demonstrates effectiveness of the ELx405 Select CW for washing cells plated into 384-well plates. Despite the cells being at a lower cell density than those shown in

96-well plates, these cells are retained after 3 cycles of washing with PBS. Using the 2x objective, the entire well can be visualized. As seen in these two representative wells, there is no loss of cells with washing at rate 11.

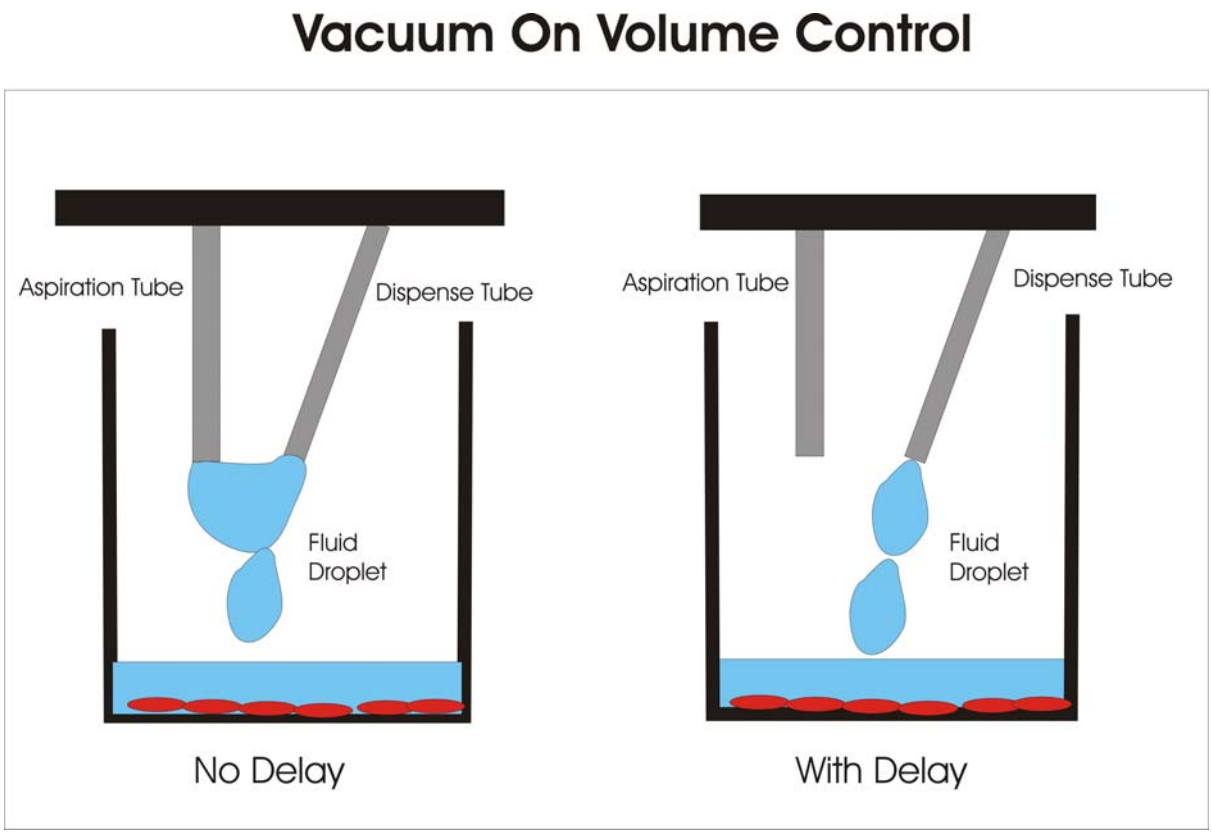

Figure 8. Illustration depicting fluid droplets with and without Vacuum on Volume delay. Droplets resulting from low flow rates have the potential to be aspirated without joining the well fluid if the aspiration vacuum is on during the dispense. With Vacuum on Volume enabled, the aspiration vacuum is delayed for a user-controlled period to allow fluid to reach the well without being aspirated prematurely.

Discussion

These observations demonstrate the utility of the ELx405™ Select CW to wash loosely adherent cells. While there are several parameters which affect the ability to reliably wash loosely adherent cells, such as BEK293T cells, the most critical parameter was fluid dispense rate. The ability of the washer to dispense fluid accurately and precisely at rates as low as 116 µl/tube/sec was critical for the maintenance of the cell monolayer. One difficulty encountered with using these low flow rates was premature aspiration of the dispense fluid. This phenomenon, as illustrated in Figure 8, results from the close proximity of the aspiration and dispense tubes. As fluid exits the dispense pipes at the lower rates, it forms droplets rather than a fluid stream. These droplets are then aspirated immediately rather than being injected into the well. To eliminate this problem, BioTek developed a Vacuum on Volume feature for the washer. This feature allows the user to delay the initiation of the aspiration volume for a brief period defined by a specific dispense volume. When the volume is achieved, the aspiration vacuum is turned on and the washer performs normally. This feature allows for the fluid droplets to flow into the well without being aspirated, yet still have overflow protection available when the aspiration vacuum is on during dispensing. Volume limitations to the parameter prevent overflow of fluid when using this command.

There are a number of suggestions in regards to washer parameter settings to optimize the effectiveness of washing cells. When selecting the dispense rate, use the highest rate possible without damaging the cells. Many cell lines tolerate normal rates without any ill effects. The faster rates will provide a more effective wash. Offsetting the dispense pipes toward the side rather than into the center of the well will dissipate the energy of the fluid stream prior to reaching the cell layer. When defining the aspiration parameters, it is advisable to leave a residual. The residual fluid will act as a shield to prevent trauma to the cell layer during the subsequent fluid dispense. It also has the added advantage of maintaining cell hydration. Avoiding aspiration delays normally used to reduce fluid residual volumes when performing ELISA is critical. There is a significant amount of vortex turbulence in the region immediately surrounding the aspiration tubes, which has the potential to be quite damaging to the cell layer.

Conclusion

The ELx405™ Select CW provides additional low flow dispense rates, effectively washing loosely adherent cell lines. Provides complete functionality of the ELx405 Select. Capable of washing cells in 96- and 384-well plates. Vacuum on Volume control prevents premature aspiration.

Acknowledgements

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