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Poster Presentation

The ELx405™ Washer

A Family of Washer to Meet the Diverse Needs of Biological Screening

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Abstract

To address the diversity of microplate washing requirements, BioTek has developed the ELx405™, a family of microplate washers that provides versatility, as well as dedicated capabilities. The ELx405 Select Microplate Washer uses a patented manifold design which provides independent control of aspirate and dispense tube location and height enabling bubble free fluid dispense and overflow protection in 96- and 384-well plates. Other models include the ELx405™ HT, which is a dedicated 384-well microplate washer, based on the same manifold design. The ELx405 Select CW was designed to wash loosely adherent cells, while the ELx405 Magna can be used for magnetic bead assays. Maintenance problems have been reduced by the introduction of the patent-pending Ultrasonic Advantage™ design, which provides ultrasonic cleaning of the manifold on most ELx405 washers. An overview of the ELx405 washer family, data demonstrating the washer's accuracy and precision, evacuation efficiency, as well as speed and timing will be included.

Introduction

The ELx405 family of washers has been designed to meet the challenges of various applications, reliability, easy maintenance, yet cost effectiveness (Figure 1). The ELx405 series of washers use BioTek Instruments' patented manifold design to overcome the difficulties presented by high density microplates and allows virtually the same functionality to be applied to the 16x24 matrix 384-well plates as that is expected with the 8x12 formatted 96-well microplate. As seen in Figure 2, the dispense and aspiration manifolds are placed into two physically different parts that are arranged on top of each other.

Figure 1. ELx405 Microplate Washer

The lower manifold (dispense) is constructed in such a manner as to allow the tube from the above 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 tilted from vertical (Figure 2.) 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

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the well from the side. This canted design also has the added benefit of providing a swirling motion of the fluid that results in a more vigorous wash.

ELx405 Select Manifold Assembly

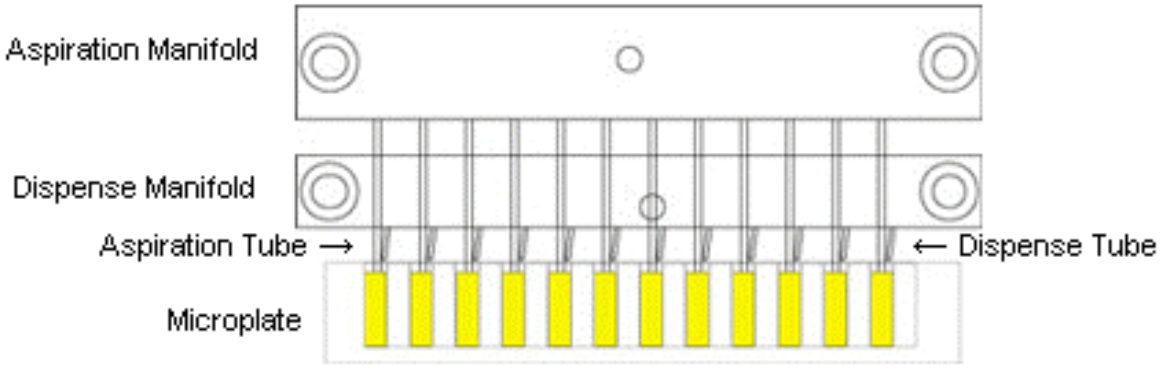

Figure 2. Schematic side view of the Washer Manifold Assembly. The aspiration and dispense manifolds are physically separated to allow for individual control of each manifold. The angled dispense pipes allow for a swirling fluid movement into the well, while allowing simultaneous access of the aspiration tube to the same well in a 384-well microplate. This unique patent pending design provides independent height control enabling bubble-free fluid dispense and overflow protection in 96- and 384-well microplates.

In order to meet the needs of a variety of different users, a family of ELx405 washers has been developed. The ELx405 Select is a multifunctional microplate washer that can wash both 96- and 384-well microplates without any mechanical changes required. This washer has 96 pairs of tubes (aspiration and dispense) that can wash all 96-wells of a 96-well plate simultaneously or wash 384-well plates in four quadrants without any manifold change. Plate type is simply selected from the software keypad. The ELx405 Magna is a 96-well microplate washer designed to wash magnetic bead based assays. By integrating strong rare-earth magnets into the plate carrier, ferrite beads can be captured as needed to allow fluid aspiration without loss. Removal of the magnet then allows complete washing of the beads. The ELx405 is a standard 96-well microplate washer for those with conventional ELISA plate washing needs. In addition to these washers, BioTek has developed washers that address specific customer needs. The ELx405 HT, which is a dedicated 384-well microplate washer that utilizes 192 pairs of aspiration and dispense pipes has been optimized for speed, to provide rapid and effective aspiration and dispensing of fluid yet have all of the features associated with 96-well plate washers for HTS customers. Where speed is essential, each complete wash cycle is run in less than 19 seconds. The ELx405™ Select CW has been designed for the most difficult cell washing applications. Many loosely attached cell lines can be disrupted by the slowest fluid dispense-rate of typical washers. 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 3). 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 extremely low rates. When standard rates are selected the flow control valve opens, allowing full fluid movement through the system. As in a standard washer configuration.

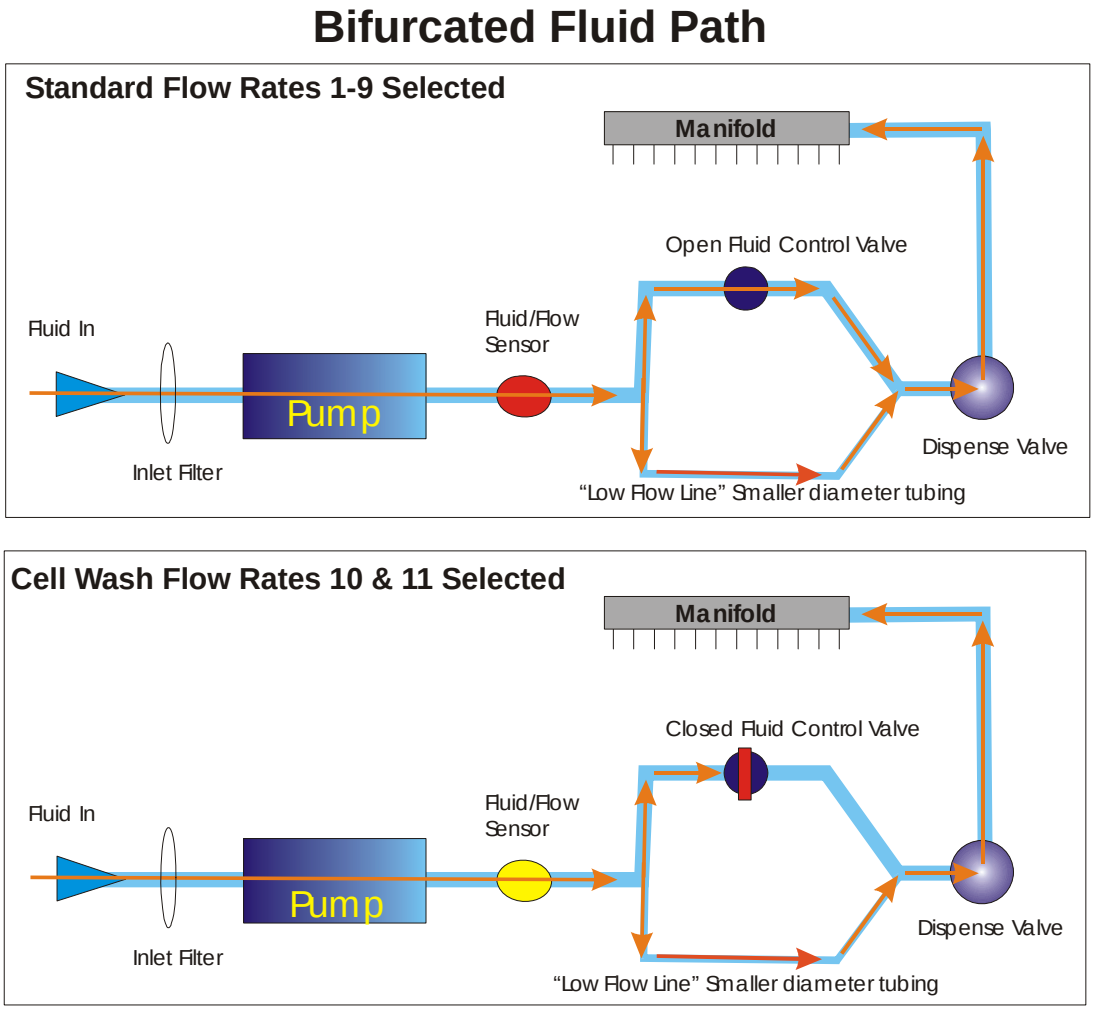

Figure 3. Bifurcated Fluid path of the ELx405 Select CW. When using standard dispense rates (rates 1-9) both pathways of the bifurcated fluid path are open. Fluid enters through the inlet filter and dispense volume is controlled by the dispense valve. If low flow rates (rates 10 & 11) are selected, the 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.

Most recently BioTek has offered an ultrasonic cleaning bath as an option to its washer line. The most common cause of washer failure and therefore "assay failure" is poor maintenance of the manifold. The use of salt and/or protein solutions can lead to formation of salt or protein deposits on the surfaces of the manifold tubes, particularly if the manifold has been allowed to dry. These deposits will reduce the orifice of the tubes and eventually clog the tubes. This phenomenon is observed as poor dispense precision and accuracy, well flooding, clogged tubes, and over all general poor washing performance. Traditionally the manifold was removed and the individual tubes were cleaned using a wire stylus or the manifold tubes were immersed in a freestanding ultrasonic cleaning bath. Ultrasonic cleaning works by creating cavitation bubbles in solutions from the energy released by ultrasonic sound waves. When the cavitation bubbles implode on surfaces, they scour any debris off the solid surface (Figure 4). With ELx405 washers that have the Ultrasonic Advantage option, the standard priming trough of the washer has been replaced with an ultrasonic bath. All of the necessary power supplies and electronics are contained within the washer without changing its footprint. This built-in feature allows the user to program automated cleaning utilities that serve to maintain manifold cleanliness without removing the manifold. Automated routines can then be programmed and used on a periodic basis in order to prevent manifold deposition.

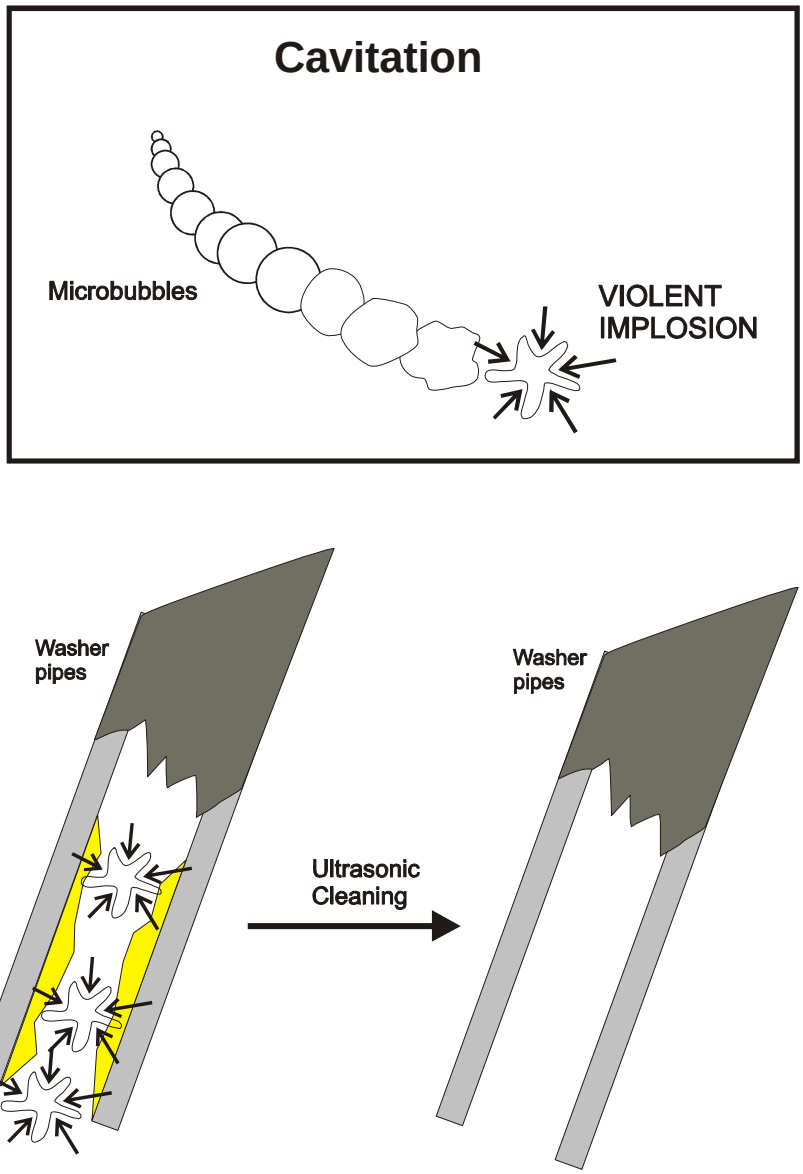

Figure 4. Depiction of ultrasonic cleaning of washer fluid tubes. The built in ultrasonic bath is energized resulting in ultrasonic sound waves that produce microbubbles as a result of cavitation. The violent bubbles collapsing along solid surfaces scour the tube surfaces clean.

Materials and Methods

QuantiGene Experiments (Branched DNA Amplification)

Cell lysates from U937 cells in volumes ranging from 0 to 20 µl were transferred to separate Capture Plate wells in replicates of eight. These lysates had been previously calibrated to be equivalent to 100 cells per 1-microliter. To each well 10 µl of GAPD pooled probe set was added. Lysis working reagent was then added to bring the total volume of each well to 100 µl. The diluted GAPD mRNA specific probe set was prepared immediately prior to use according to the kit instructions. Briefly, 5X-prepackaged probe sets (100 nm LE, 500 nM BL, and 250 nM CE) were diluted to 1X using TE (10 mM Tris 1 mM EDTA pH 7.5-8.0) buffer. A pooled probe set

was then prepared by adding 200 µl of each probe set to 3400 µl of undiluted lysis mixture. The Capture Plates were sealed and incubated at 53°C for 16-20 hours (overnight).

The following day wells were washed either manually or with an ELx405™ Select Microplate Washer (BioTek Instruments) as described in the washing instructions below. Wash buffer consisted of 0.1X saline-sodium citrate buffer (SSC) and 0.3% lithium lauryl sulfate in distilled H_2O. After washing 100 µl of Amplifier working solution was added to each well and the plates were resealed and incubated for 60 minutes at 46°C. Amplified working solution was prepared immediately prior to use by diluting amplifier stock solution with amplifier/label probe diluent (both supplied in the QuantiGene kit) in a ratio of 1:1000 (stock:diluent). After incubation, the plates were again washed either manually or with an ELx405 Select and 100 µl of diluted label probe solution was added. The diluted label probe solution was prepared immediately prior to use by the dilution of label probe concentrate with amplifier/label probe diluent in a ratio of 1 µl concentrate to 1 ml of diluent (both supplied in the QuantiGene kit). The plates are again incubated for 60 minutes at 46°C. Following the second incubation plates were rewashed. After the final wash, working luminescent alkaline phosphatase dioxetane substrate was added, the plate was sealed and then incubated for 30 minutes at 46°C. Working substrate solution was prepared by adding 3 µl of a 10% lithium lauryl sulfate solution each 1 ml of the stock luminescent substrate. The luminescence signal was determined using a luminometer set at 41°C.

Cell Washing Experiments
HEK293T cells were cultivated in DMEM (10% FCS) and plated into 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.

ELx405 HT Performance Experiments
The absorbance of a dye solution (FD&C Blue No. 1) was used to estimate the dispense precision of the ELx405™ HT Microplate Washer in a manner similar to that described previously. The dispense-volume accuracy into 384-well microplates was determined by weighing an empty plate before and the same plate after the dispense cycle by the ELx405 HT washer. The average weight per well was calculated by dividing difference between initial and final weights (Delta) by the total number of wells (384). Using the specific gravity of water (1 g/ml) a conversion from weight to volume was then made. Next, deionized water was added to each well of the microplate such that the final volume would be expected to be 110 µl. Note that the volume added varied depending on the intended dispense-volume programmed. The absorbance at 630 nm (450 nm reference) of all the wells in a 384-well microplate were measured using a Synergy™ HT Multi-Detection Reader (BioTek Instruments) and the average calculated. A plate-specific factor was then calculated by dividing the average per-well absorbance by the per-well dispense volume. This factor was then used as a conversion factor to calculate the dispense volume of each well from its absorbance. Residual determinations were accomplished using a gravimetric method and were performed by weighing dry empty plates using a Sartorius A 120S analytical balance. After weighing, 100 µl of fluid was added to the wells of each plate and the ELx405 HT washer was used to aspirate the fluid. After

aspirating the fluid, the plate was quickly re-weighed and the resultant weight change, when divided by the number of plate wells, returned an average per-well dispense volume.

Results

Different experiments were run to demonstrate the performance and utility of some of the washers. For example, the ELx405 Select was used to perform the wash steps necessary with the QuantiGene Reagent System from Genospectra. This assay system employs branched DNA technology to amplify the signal rather than the target for direct RNA quantitation in mixed samples. Figure 5 demonstrates the intra-assay repeatability of the QuantiGene Reagent system when using the ELx405 washer. There is very close agreement of luminescent between individual wells when four different volumes of cell lysate are assayed. In this experiment a multichannel pipettor was used such that all eight wells of a strip were pipetted with the same barrel. Differences within the strip would be the result of the washer, while differences between strips is most likely the result of pipettor error. Note that while the overall signal for the 20 µl-lysate in the last data set in Figure 6 is slightly lower than the other two corresponding data sets, the individual values are all in close agreement, with CVs of less than 4%. On average the CVs for all the strips of the experiment were approximately 5%. When the detection limits of the QuantiGene Reagent system are examined, both the manual and the automated wash procedure produce similar results. When the signal to noise ratio of the data depicted in Figure 6 is calculated for the lowest concentration measured (0.04 attomoles) a value greater than 7 is returned for the samples washed using the ELx405. Values greater than 2 are considered significantly different than the blanks. As demonstrated in Figure 7, measurement of GAPDH from total RNA is quantitative and linear for both the manual and automated wash methods. When using total RNA as the target, this technology can detect GAPDH from as little as 18 ng of total RNA without having to amplify the target. At this level the signal to noise ratio of the measurement is greater than 25 (data not shown), which suggests that levels much lower could reliably be detected.

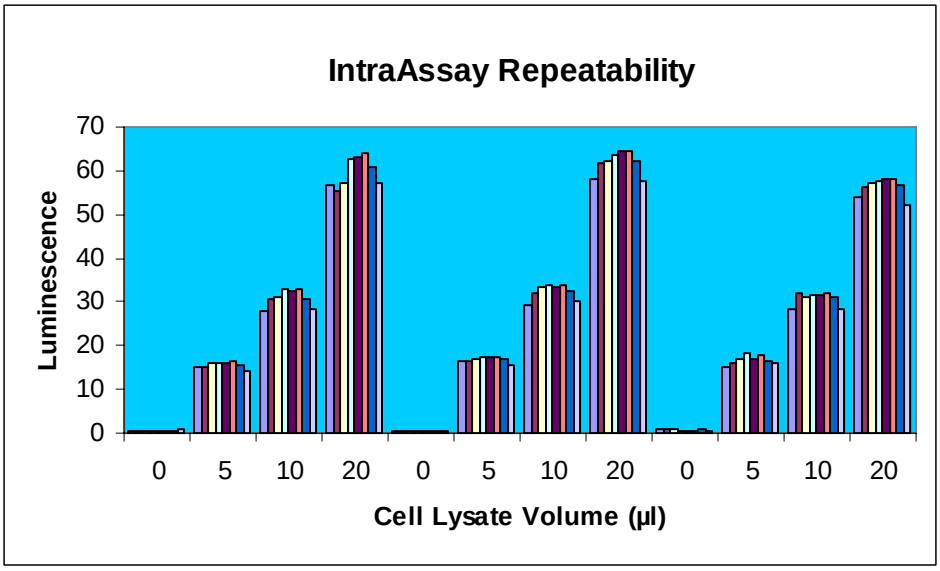

Figure 5. Intra-assay Repeatability of QuantiGene Assay using the ELx405 Washer. Indicated volumes of cell lysates were assayed with the QuantiGene assay system using the ELx405 washer to automate the wash steps. Each grouping represents a single strip of the microplate pipetted with the same barrel of a multichannel pipette, while each bar represents the result of an individual well of the microplate.

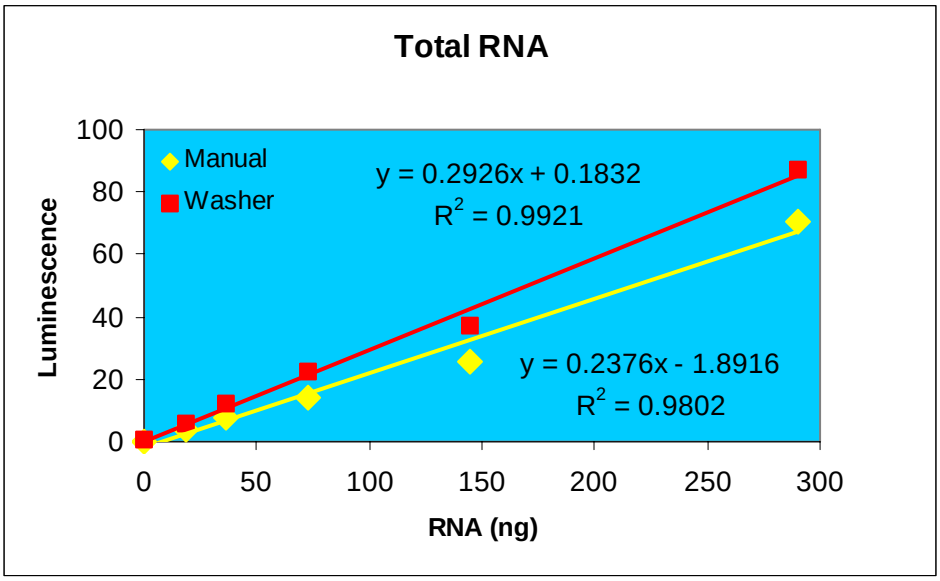

Figure 6. Total RNA Quantitation Using the QuantiGene Reagent System washed manually or using the ELx405. RNA samples ranging from 0 to 290 ng of total RNA were assayed for GAPDH in parallel where one plate was washed manually while the other was washed using the ELx405 Select Microplate Washer.

When the ELx405™ Select CW was used to wash loosely adherent cells, the utility of the slow flow rates becomes apparent. As demonstrated in Figure 7, 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 7B and 7D) 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. When the low flow rate 11 (116 µl/tube/sec) is enabled there is virtually no change in regards to cell number or morphology when washing HEK293T cells. When the images from two different wells obtained before (Figure 8A and 8C) and after (Figure 8B and 8D) 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.

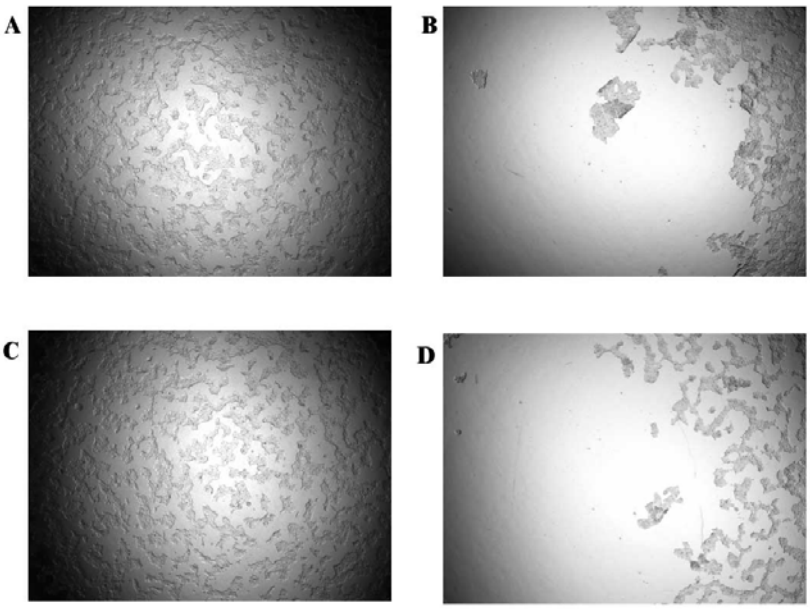

Figure 7. Before and after washing 96-well plate with cells using standard dispense rate. Two different wells of a 96-well plate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2X objective.

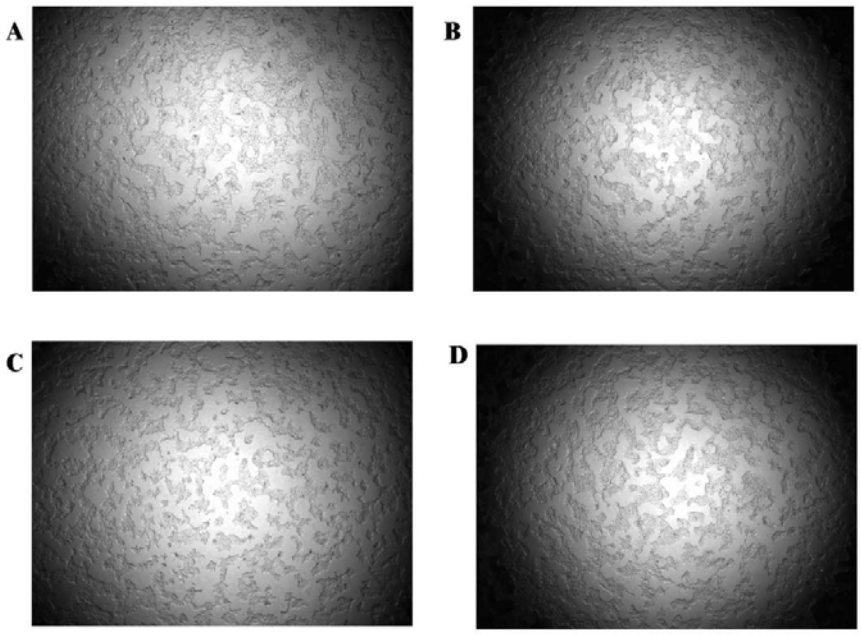

Figure 8. Before and after washing cells using low flow rate setting. Two different wells of a 96-well plate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2X objective.

The accuracy and precision of dispense for the ELx405™ HT was determined using a combination of gravimetric and colorimetric methodologies. The dispense accuracy was determined for volumes ranging from 10 to 120 µl gravimetrically. For each of the indicated volumes an empty microplate was weighed before and after a dispense routine was run. Thus the data for each plate represents the mean of 384-wells. The percent error was found to be 8.5% at the lowest expected volume tested (10 µl) (data not shown) and decreased to 1% or less at volumes above 50 µl (Fisgure 9). Note that volumes less than the recommended lower limit of 25 µl (as indicated on the washer programming keypad) can be run. While these volumes are not necessarily recommended, they are available to the end user, with the caveat that performance may not be guaranteed. The slight increase in the percent error seen at 30 and 50 µl may be, in part, the result that only one plate per data point was measured. Because the solution used for the dispense-accuracy determinations contained a light absorbing dye (FD&C Blue No. 1) the absorbance of each well was used to determine dispense precision. The coefficient of variance (%CV) was found to be approximately 8% at 10 µl per well. The %CV also decreased rapidly with larger volumes to less than 4% at volumes above 30 µl per well. As mentioned previously, volumes less than 25 µl are not recommended, but can be utilized with the washer. Despite this, lower volumes provide generally acceptable results. Difficulties with a 10 µl-dispense are not surprising when one considers the requirement of moving these very small amounts through 192 dispense tubes. Another important parameter with microplate washers is residual volumes. As demonstrated in Figure 11, use of the 192-tube manifold provides very similar residual volumes as found in the 96-tube manifold. The residual volume for three different 384-well plates averaged less than 0.6 µl per well, with the maximal well averaging about 1.2 µl for the three plates. This compares quite favorably to the data obtained when the

ELx405 Select manifold (96-tubes) was used. These data suggest that use of the 192-tube manifold should provide equivalent washing capabilities in 384-well plates as found in the industry standard ELx405 Select 96/384-well plate washer.

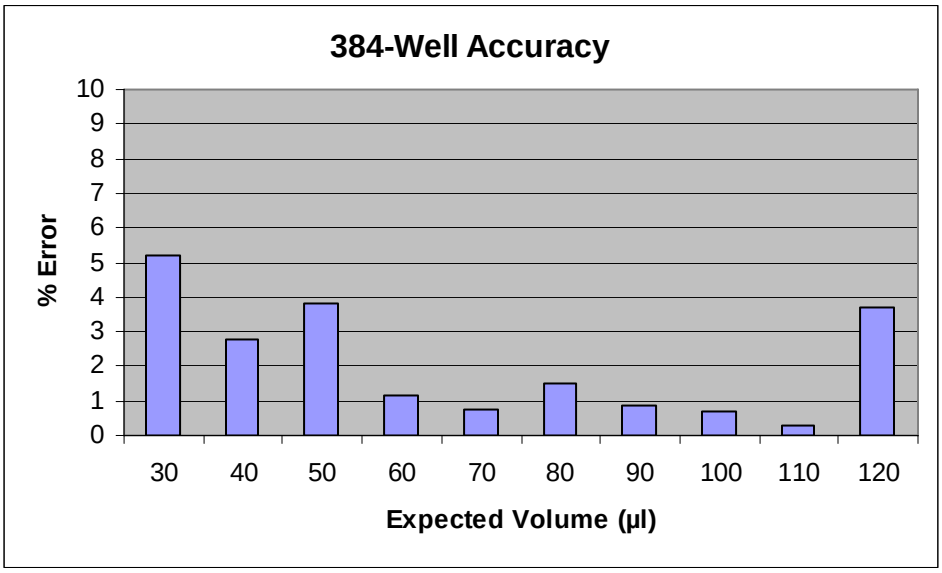

Figure 9. Dispense Accuracy of the ELx405™ HT Microplate Washer. For each volume indicated, individual 384-well microplates were weighed. After dispensing the indicated volume using the ELx405 HT, the plates were re-weighed. The total residual volume of the plate was calculated assuming a specific gravity of 1 g/ml. The percent deviation is the ratio of the difference between the calculated and expected values to the expected values expressed as a percent.

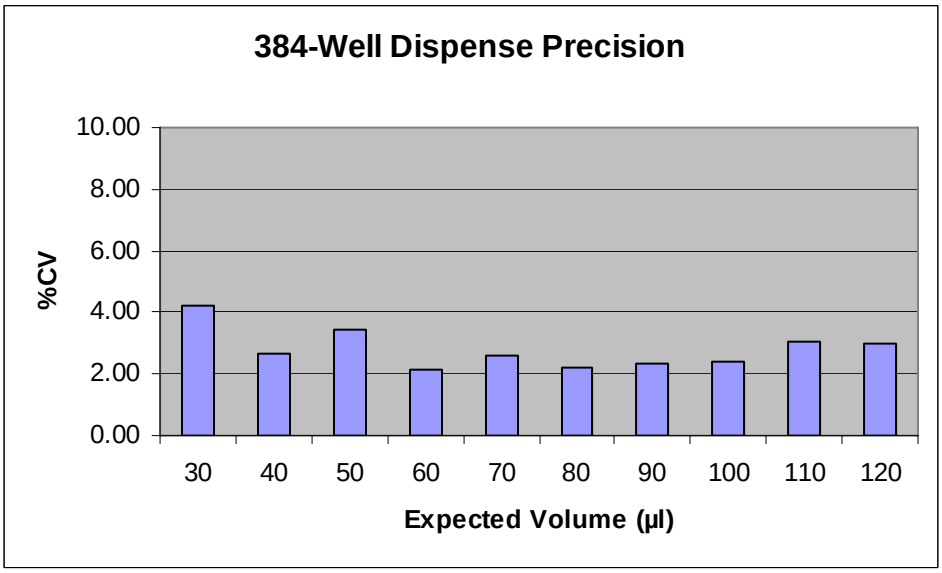

Figure 10. Dispense Precision of the ELx405 HT. The volume dispensed to each well was determined as described in the materials and methods. The use of a plate-specific conversion factor that allows the conversion of an absorbance measurement to volume. The mean, standard deviation and %CV were then calculated from the calculated volumes. Note that the %CV for each indicated volume represents 384-well determinations.

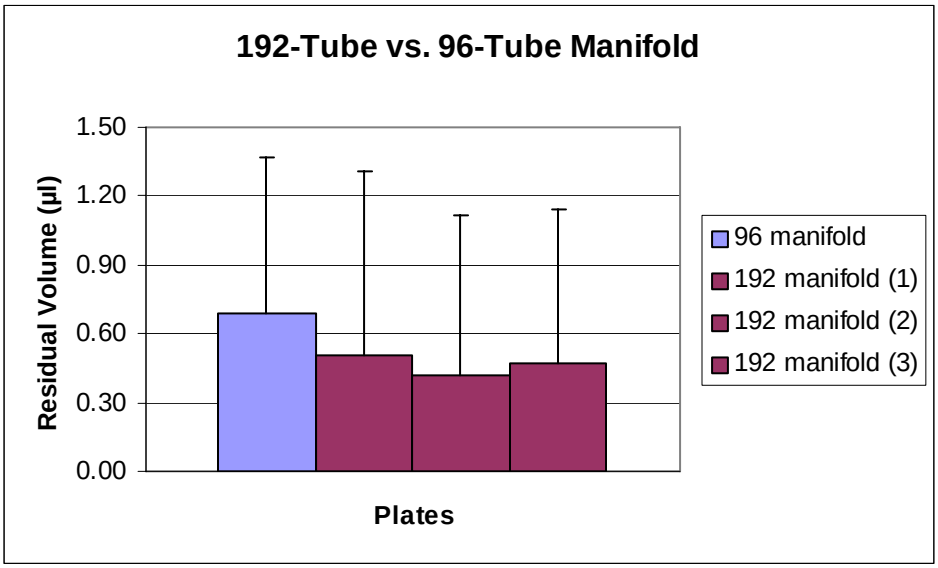

Figure 11. Comparison of 96-well and 192-well manifold Residual Volumes. Using the same ELx405 Washer, 384-well plates containing 100 µl of concentrated blue dye were aspirated with either the standard ELx405 Select 96 tube-pair manifold or the ELx405 HT, 192 tube-pair manifold, were tested for residual liquids as described in materials and methods. Note that error bars and represent the maximal residual volume in any well.

Summary

(1) The ELx405 Microplate Washer offers a wide range of full plate 96- and 384-well washer options

(2) All ELx405 Washers are based on the same rugged dependable design

(3) The ELx405 Washer family is capable of a wide variety of tasks

(4) The ELx405 Washers are robotics compatible

(5) The ELx405 Washers are Accurate and Precise over their entire range of volumes

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