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Poster Presentation

The ELx405™ 384-Well Microplate Washer

Designed to Meet the Rigors of Biomoecular Screening

Authors: Paul Held and Lenore Buehrer, BioTek Instruments, Winooski, Vermont

Abstract

In today's HTS environment, 384-well microplates have become the preferred format, regardless of the screening assay type. Most often these assays require some sort of aspiration and/or dispensing of fluids to the wells of the microplate, which are typically accomplished by a microplate washer. The ELx405™ HT is a dedicated 384-well microplate washer that utilizes 192 pairs of aspiration and dispense-tubes to provide rapid and effective aspiration and dispensing of fluid without sacrificing features or performance. Using the same patented dual-action manifold design as the industry standard ELx405™ Select washer, the ELx405™ HT allows independent control, both vertically and horizontally, of the aspiration and the dispense manifolds. Several standard features have been included to make the ELx405 washer robotic-friendly and ideal for automation. Vacuum detection is provided to ensure that the vacuum pump is switched on and vacuum vessels are connected. Flow protection alerts the user to an interruption of flow to the dispensing manifold. Waste-level detection prevents overflow of waste into the vacuum pump. In addition to an overview of the ELx405™ HT washer, data demonstrating the washer's accuracy and precision of fluid-dispense, evacuation efficiency, as well as speed and timing, will be included.

Figure1. The ELx405HT 384-well microplate washer.

Introduction

Regardless of the screening assay type or size, microplates are the preferred format, with high throughput screening (HTS) assays primarily using 384-well microplates. Most often these assays require some sort of aspiration and/or dispensing of fluids to the wells of the microplate which is accomplished by a microplate washer. Towards that end, BioTek showcases the ELx405 HT washer (Figure 1), which joins a family of microplate washers that have become the industry standard. The ELx405 HT is a dedicated 384-well microplate washer that utilizes 192 pairs of aspiration and dispense-pipes to provide rapid and effective aspiration and dispensing of fluid yet have all of the features associated with 96-well plate washers. Other washers in the ELx 405 family include the ELx405 Select, the ELx405 Select Cell washer, the ELx405 Magna, and the ELx405. The ELx405 Select is a multifunctional microplate washer that can wash both 96- and 384-well microplates without any mechanical changes required. This plate washer uses a patented manifold design which provides for independent control of aspirate and dispense tube location and height enabling bubble free fluid-dispense and overflow protection in 96- and 384-well plates. The ELx405 Select CW offers all of the features of the ELx405 Select in addition to being optimized fro cell washing. 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. And finally, the ELx405 is a standard 96-well microplate washer for those with conventional plate washing needs. This washer is a direct descendant from a long line of BioTek plate washers that have provided tremendous performance for years. Here we describe several performance tests that demonstrate the capabilities of the ELx405 HT washer in regards to addressing the HTS needs.

Materials and Methods

The reagents required to perform these studies were obtained from several different vendors. Phosphate buffered saline (PBS) tablets (catalogue no. P-4417) were obtained from Sigma Chemical Company (St. Louis, MO). The 384 well microplates (catalogue number 242757) were from Nunc (Naperville, IL), and FD&C Blue dye #1 powder was obtained from Prime Ingredients (Saddle Brook, NJ).

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. In addition, the absorbance of a concentrated blue dye solution was used to estimate the residual fluid remaining in each well of a microplate. The total residual volume from 384-well microplates was determined by weighing an empty plate before and after the aspiration cycle by the ELx405 HT washer. The average per-well residual was calculated by dividing the 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, 80 µl of deionized water was added to each well of the microplate and the absorbance at 630 nm (450 nm reference) of all the wells in a 384-well microplate were measured and the average calculated. A plate-specific factor was then calculated by dividing the average per-well absorbance by the per-well residual volume. This factor was then used as a conversion factor to calculate the residual volume of each well from its absorbance. Mean values, as well as maximum and minimum per volumes, were then determined from the converted data.

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 the 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 of it 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. Mean values, as well as maximum and minimum per volumes, were then determined from the converted data.

Washer efficiency was determined using the activity of residual amounts of horseradish peroxidase (HRP) enzyme activity. Prior to washing, 50 µl of a HRP stock solution (100 µg/ml in goat serum) was pipetted to each well of a 384-well microplate using a Precision Automated Pipetting System (BioTek Instruments). Filled plates were then immediately washed with the phosphate buffered saline (PBS) with 0.1% Tween 20 wash buffer for the indicated number of cycles (100 µl/well dispense volume) with a final aspiration. After the completion of the wash routine, the ELx405 HT washer was used to dispense 40µl of PBS with 0.1% Tween 20 to all the wells. HRP activity assay was initiated by adding 40 µl of a TMB/H_2O_2 substrate solution and incubated at room temperature for 30 minutes. Note that the TMB/H_2O_2 solution was prepared immediately prior to use by mixing equal volumes of TMB peroxidase substrate and peroxidase solution B solutions from Kirkgaard & Perry Laboratories (Gaithersburg, MD). The reaction was stopped by the addition of 20 µl 1N H_2SO_4 and HRP activity assessed by measuring the absorbance of each well at 450 nm (630 nm reference) in a Synergy HT Multidetection microplate reader (BioTek Instruments, Winooski, VT). HRP activity was then calculated by using the absorbance values to interpolate a previously prepared calibration curve.

Results

The time necessary to perform routine washing protocols by the ELx405 HT was examined using a variety of wash parameters. Using protocols with different numbers of wash-cycles in conjunction with various dispense-volumes and different speed groupings, which correspond roughly to slow, medium, and fast settings, the time needed to accomplish the wash methods was measured. At the fastest setting the ELx405 HT can perform a 1-cycle wash (100 µl dispense) of a 384-well microplate in less than 18 seconds and 300-µl wash in less than 20 seconds (Table 1). This time includes the time required to move the plate carrier from the home position to the manifold at the beginning of the wash cycle as well as returning to the home position at the completion of the cycle. The time necessary to accomplish the wash routine is dependent on a number of parameters. There are a total of 9 dispense rate settings, ranging from 102 to 209 µl/tube/sec, and 10 aspiration dive rate settings ranging from 3.0 to 15 mm/sec. In addition the dispense volume can be varied up to 3000 µl. All three of these parameters can be controlled independently to achieve optimal washer performance. As one might imagine, with larger per-well volumes, as well as increasing numbers of cycles, the time required to wash a plate increases. As demonstrated in Table 1, the time necessary for 300 µl wash with one cycle is 37.44 seconds at the slowest settings, while five cycles at the same settings required 120 seconds. Likewise the time required to wash 5 cycles with a 25 µl dispense volume at the fastest setting is 46.47 seconds; when the volume is increased to 300 µl the ELx405 HT requires 60.93 seconds.

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 110 µl gravimetrically. For each of the indicated volumes an empty microplate was weighed before and after a dispense routine, and was run at the slowest rate. Thus the data for each plate represents the mean of 384-wells. As demonstrated in Figure 2, the percent error was found to be 8.5% at the lowest expected volume tested (10 µl) and decreased to 1% or less at volumes above 50 µl. Note that the volumes of 10, 15 and 20 µl are less than the recommended lower limit of 25 µl as indicated on the washer programming keypad. 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. As demonstrated in Figure 3, 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 volumes through 192 dispense tubes. The linearity of dispensing was examined by comparing the calculated average per-well-dispense to the expected volume. As shown in Figure 4, the relationship between calculated and expected is very close to ideal. While a perfect match would result in a linear regression that passes though the origin and has a theoretical slope of 1.00, these data indicate a slope of 0.989 with a correlation coefficient (r^2) of greater than 0.999.

Another important parameter with microplate washers is residual volumes. As demonstrated in Figure 5, 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. This is further demonstrated by Figure 6, which shows the wash efficiency of the ELx405 HT with increasing wash cycles. After 2 cycles, the concentration of HRP in the wells has been reduced from 100 µg/ml to approximately 60 pg/ml and after three cycles the residual amount of HRP has been reduced to 10 pg/ml. This represents a decrease of 1 $x10^7$ fold. HRP activity is almost undetectable after 5 wash cycles.

Discussion

Today's HTS procedures require that instrumentation be cost effective, reliable and easy to use. BioTek Instruments' answer to these needs is the ELx405 HT washer. The ELx405 HT's manifold has a unique patented design which provides for independent control of aspirate and dispense tube location and height enabling bubble free fluid-dispense and overflow protection in 384 well plates. The manifold allows for overflow protection, as well as "bottom washing" and overwashing a well with more volume than it can hold for improved wash capability.

These data presented demonstrate that the ELx405 HT 384-well microplate washer provides the performance necessary to address the needs of the HTS market. The ELx405 HT uses the same patented manifold design as the standard ELx405 Select. Washer. This manifold design offers several advantages over other designs. Other designs which have been used to address the small size of the 384-well format include (1) a tube within a tube with the dispense pipe centered inside the aspiration pipe; (2) separate adjacent aspiration and dispense manifolds; and (3) traditional side by side pipes. The tube in a tube design's chief advantage is the ability to position the tube at several locations close to the sides of the well for efficient evacuation. This procedure is often referred to as a "bottom sweep". The tube in a tube design, which allows for simultaneous access of the aspiration- and dispense-tubes to the same well, theoretically provides for overflow washing. In practice, this ability is limited due to the possibility of suctioning the fluid directly out of the dispense pipe into the aspiration pipe before it gets to the well. This problem is particularly evident with low fluid delivery rates and small 384-well plates. Conventional side-by-side pipes have also been modified to accommodate 384-well microplates. In general, this modification has been to shorten the dispense-pipe to the height of the microplate. Unfortunately this results in considerable splashing of the liquid being dispensed. The use of separate aspiration and dispense manifolds in two locations solves the splashing problems and also allows for "bottom sweep" aspiration, but cannot offer overfill washing because the two manifolds do not access the well at the same time. BioTek's patented design alleviates these problems and provides for the same functionality in the 384-well format as has become expected in the 96-well format. While it as been specifically designed for use with 384-well plates, the choice of 192 tube pairs rather than 384 provides as many advantages in terms of maintenance and fluid delivery. The physical demands in terms of the necessary small tube bore size with 384 tube pairs results in difficulties with priming and clogging. The large amount of tube surface associated with 384 tube pairs requires large volumes of buffer for priming to insure that all the tubes have been cleared of air bubbles. The small diameter of tubing (both aspiration and dispense) results in more clogging. Because the ELx405 HT uses 192 tube-pairs with larger bore tubes, clogging and priming problems are minimized. Despite half the numbers of tube-pairs the ELx405 washer provides over all wash times equivalent to competitor's 384-tube washers.

Several standard features have been included to make the ELx405 HT washer robotics friendly and ideal for automation. Features, such as vacuum detection, are provided to ensure that the vacuum pump is switched on and vacuum vessels are connected. Flow protection alerts the user to an interruption of flow to the dispensing manifold. The washer has a waste detection device, which through software prevents overflow of waste into the vacuum pump. Because buffer is drawn from reservoirs by suction from a diaphragm pump, pressurized buffer containers are not required for operation. This affords the user the opportunity to use any size reagent container they choose. An optional buffer-switching module allows for the automatic switching from up to four different buffers. The washer also has a separate priming trough to allow for automatic priming ("autoprime") and automatic rinsing ("autorinse") during wash procedures. The ELx405 HT has been designed to be robotics friendly, using an ActiveX programming, which provides easy system integration and RS-232 connections that allow communication with most robotics devices. BioTek also manufactures the Bio-Stack, a dedicated plate stacker designed to interface with the ELx405 series of washers, as well as a number of other devices.

The ELx405 HT washer can be programmed directly from its keypad on the washer with full functionality. Assay libraries containing as many as 75 different assays can be stored on the washer. The ability to link different wash, prime, and dispense protocols allows for virtually an endless variety of washer protocols.

Many different safety features have been incorporated in the design of the washer, including the separation of the electronic circuitry from the fluidics. It conforms to electrical safety standards, EN61010, UL3101-1, and CAN/CSA C22.2 No. 1010.1. The ELx405 also meets the CE mark. Besides being versatile, the washer is lightweight (<13.5Kg), compact (41x41x26 cm) and capable of 110- and 220-volt operation.

Table 1. Wash Times* for the ELx405 HT at Different Rate Settings:

#Flow rate=1, &Aspiration rate=1			
	Wash Volume (µl)		
# Cycles	25 µl	100 µl	300 µl
1	32.04	33.47	37.44
2	46.99	50.14	58.44
3	62.14	66.9	79.22
4	77.22	83.5	99.97
5	92.6	100.33	120.69
10	167.9	183.47	224.5
Flow rate=5, Aspiration rate=3			
	Wash Volume (µl)		
# Cycles	25 µl	100 µl	300 µl
1	26.94	28.47	31.26
2	39.56	42.45	47.8
3	51.98	55.77	63.84
4	64.8	69.27	80.32

5	77.21	82.86	96.55
10	140.07	150.95	178.09
Flow rate=9, Aspiration rate=6			
	Wash Volume (µl)		
# Cycles	25 µl	100 µl	300 µl
1	16.71	17.97	19.52
2	24.08	25.81	29.9
3	31.6	34.1	40.36
4	38.97	42.27	50.52
5	46.47	50.65	60.93
10	83.48	91.6	112.36

*Times indicated are the time required to wash the wells of a 384-well microplate using the indicated dispense volumes and number of wash cycles in seconds. (This includes moving the plate from the rest position to the wash head and back.)

[#] Flow rate settings of 1, 5, and 9 correspond to flow rates of 102, 162, 209 µl/tube/sec, respectively.

[&] Aspiration rate settings of 1, 3, and 6 correspond to dive rates of 3.0, 4.0, and 15.0 mm/sec respectively.

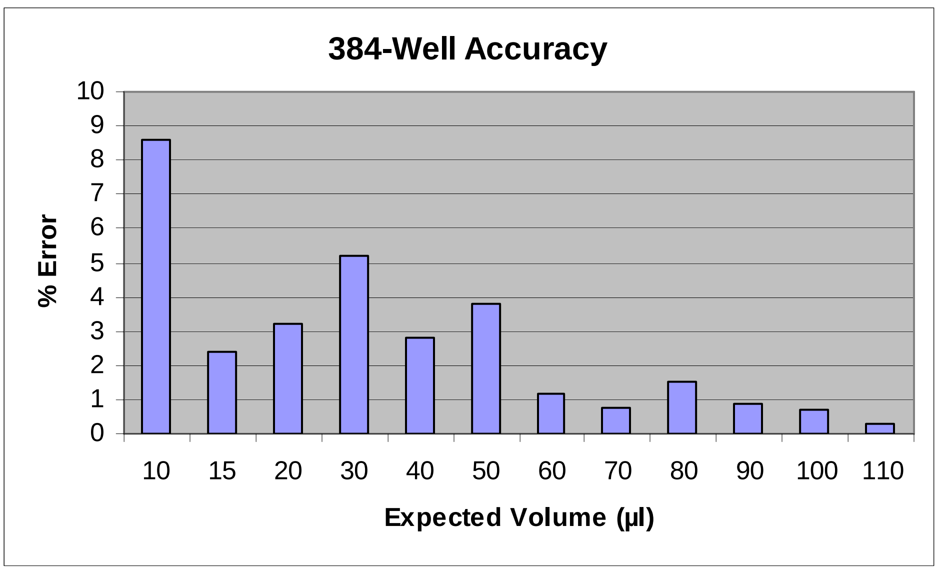

Figure 2. Dispense Accuracy of the ELx405 HT Microplate Washer. For each volume indicated, individual 384-well microplates were weighed. After dispensing the indicated volume using the ELx405 HT, the plates were re-weighed. The total residual volume of the plate was calculated assuming a specific gravity 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.

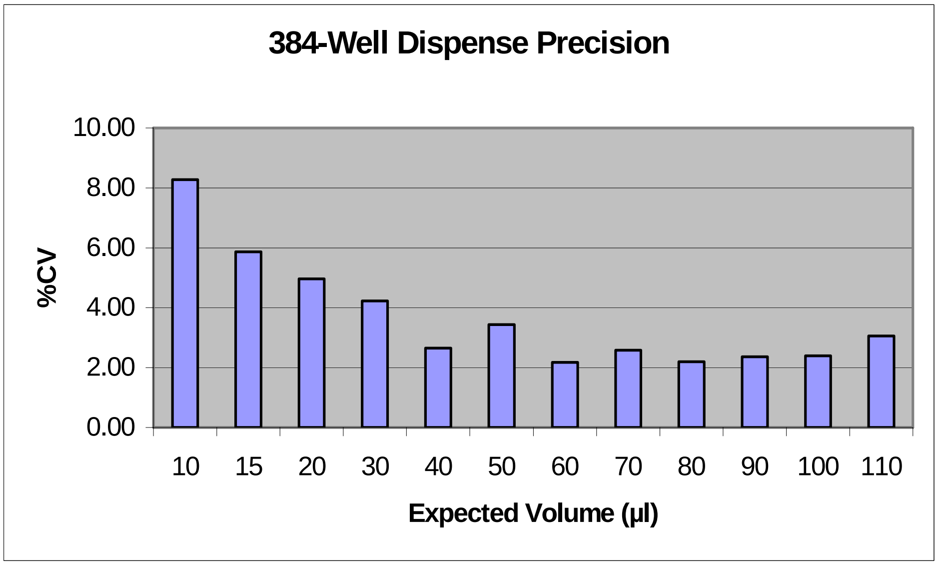

Figure 3. Dispense Precision of the ELx405 HT. The volume dispensed to each well was determined as described in the materials and methods. The use of a plate-specific conversion factor that allows the conversion of an absorbance measurement to volume. The mean, standard deviation and %CV were then calculated from the calculated volumes. Note that the %CV for each indicated volume represents 384-well determinations.

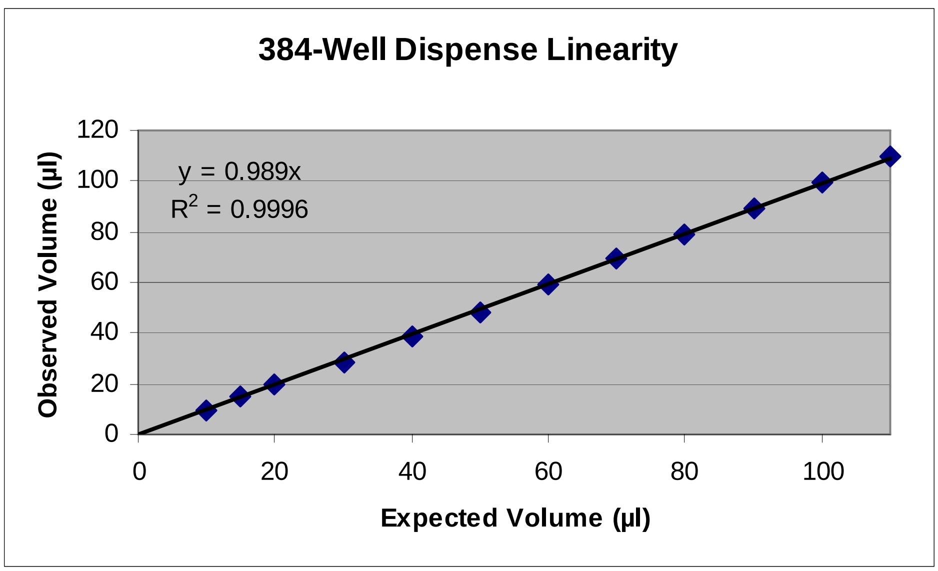

Figure 4. Linearity of Dispense. The calculated observed volume from data presented in Figure 3, was plotted against the corresponding expected volume. The data (blue diamonds), which represent the mean determination of 384–wells, was plotted and a linear regression analysis performed. The subsequent regression (solid line) and its equation and correlation coefficient are also presented.

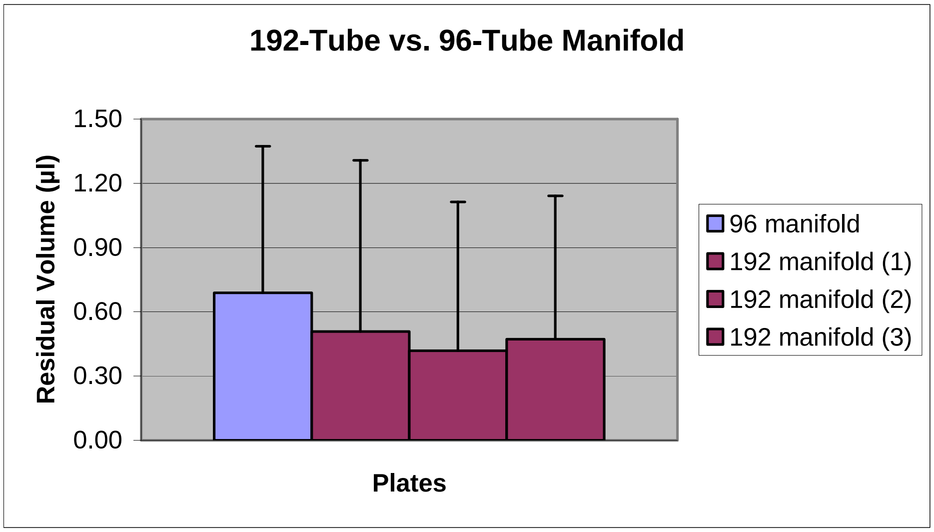

Figure 5. Comparison of 96-well and 192-well manifold Residual Volumes. Using the same ELx405 washer, 384-well plates containing 100 µl of concentrated blue dye were aspirated with either the standard ELx405 Select 96 tube-pair manifold or the ELx405 HT, 192 tube-pair manifold, were tested for residual liquids as described in materials and methods. Note that error bars represent the maximal residual volume in any well.

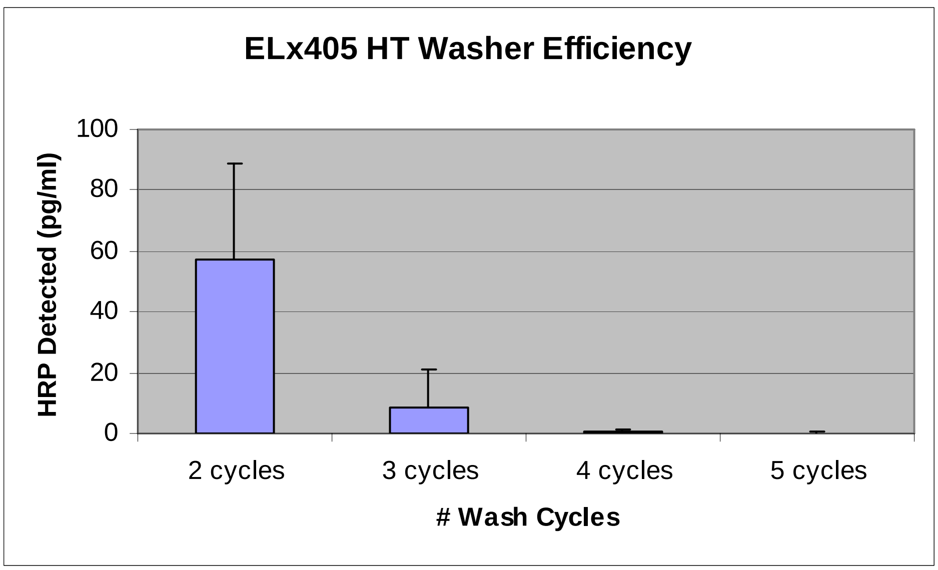

Figure 6. ELx405 HT Washer Efficiency. Horseradish peroxidase (HRP) enzyme was pipetted into all the wells of 384-well microplates and the plates washed with the indicated numbers of wash cycles using an ELx405 HT Microplate Washer. HRP activity was assayed after washing and activity calculated by comparison against a previously prepared standard curve. Note that each bar represents the mean and standard deviation of all the wells of a 384-well microplate.

Summary

The ELx405 HT Microplate Washer provides all of the functionality of the ELx405 Select along with increased speed when washing 384-well microplates.

- The ELx405 HT provides equivalent performance in terms of accuracy and precision as the ELx405 Select
- The ELx405 HT uses the same-patented manifold design as the industry standard ELx405 Select
- The ELx405 HT provides improved speed when washing 384-well plates
- The ELx405 HT is robotics compatible

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