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Poster Presentation

µFill™

A Microplate Reagent Dispenser for HTS and Drug Discovery

The µFill is a reagent dispenser capable of dispensing to either 96- or 384-well microplates without any hardware changes and 24-well plates with a manifold change. Additionally, the µFill is capable of dispensing to deep-well microplates by changing the plate carrier. In this monograph, we will discuss the µFill's speed, accuracy, and precision at fluid volumes that span its reported range. The µFill is provided as two different models (standard and autoclavable) to meet the diverse needs of today's investigator. Besides the standard model, BioTek has developed the autoclavable model in response to the need for easy sterilization of the µFill's fluid path. The syringe barrel and piston have been located externally, and along with the tubing, check valves and manifold have been designed to be fully compatible to autoclaving. This allows the user to steam-sterilize the entire fluid path instead of using chemical sterilization methods prior to dispensing sterile solutions. Additionally, the autoclavable version of the µFill offers increased organic resistance. Using the newly designed manifold and syringe pump in conjunction with optional chemical-resistant check valves allows the µFill to be used with agents such as dimethyl sulfoxide (DMSO) and acetonitrile.

Introduction

Figure 1. µFill 96-/384-Well Microplate Dispenser.

Today's biomedical research requires instrumentation that is both functional and versatile. While high throughput screening (HTS) and drug discovery laboratories require instrumentation that can be automated, pilot assay laboratories may not necessarily need total automation. Towards that end, BioTek has developed the µFill, (pronounced "micro" Fill) reagent dispenser capable of running stand-alone or computer controlled as part of a robotics system (Figure 1). The µFill is compatible with both conventional 96- and 384-well microplates, and by removing the standard plate holder, can also dispense into deep-well microplates. The use of a specialized 8-channel manifold also allows the µFill to be used with half-area 96-well plates and 24-well plates. The microprocessor - controlled syringe pump is based on a tested, low maintenance design that requires no recalibration, yet provides a high degree of accuracy and precision. As part of the manufacturing process, BioTek performs a 6-point calibration procedure to accurately tune the syringe pump over a wide range of volumes. Among the many variables which programming allows, is the control of flow rates from (225 µl/well/sec), for dispensing to cell cultures, to (1000 µl/well/sec) for rapid and vigorous reagent dispensing. The flexible

software provides complete programming capabilities from the keypad. For more complete automation, robotic interfaces can be developed using ActiveX® software commands. The µFill's diminutive size, with a 14 x 14-inch footprint and a height of 7 inches, allows it to be used almost anywhere.

Materials and Methods

Dispense accuracy and precision was determined using a combination of a gravimetric method and the absorbance of dye solutions. Gravimetric determinations were performed by weighing plates using a Sartorius A 120S analytical balance, while the absorbance of a dye solution (FD&C Blue No. 1) was used to estimate the dispense precision of the µFill Microplate Dispenser. The dispense-volume accuracy into 96- or 384-well microplates was determined by weighing an empty plate before and the same plate after the dispense cycle by the µFill. The average weight per well was calculated by dividing difference between initial and final weights (Delta) by the total number of wells (96 or 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 was expected to be 300 µl or 100 µl for 96-well and 384-well plates respectively. 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 the 96- or 384-well microplate was measured using a Synergy HT Multidetection 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.

Tests involving the use of Sephadex beads were done as follows. Briefly, a mixture of deionized water and hydrated Sephadex G-50 Superfine beads (Amersham Pharmacia Biotech, Piscataway, New Jersey) was stirred using a magnetic stirrer at a setting sufficient to prevent the slurry from settling. The aspiration tubing was situated such that the end was located approximately 2.5 cm (1 inch) from the bottom of the reservoir. It was found that maintaining the slurry in an agitated state was paramount to accurate dispensing of the beads. If there was a delay of greater than 10 minutes between plates, the µFill was re-primed using the "New Buffer Prime" software selection. Note that beads lost through priming can be saved using the drain hole and tubing to a collection container. All gravimetric experiments were performed as described previously. It was also observed that light scattering by the beads could be used to measure the volume of bead dispense optically. While several different wavelengths were tested, 405 nm appeared to provide the most reliable results. Using a µQuant microplate spectrophotometer (BioTek Instruments, Winooski, VT), the absorbance of samples at 405 nm were measured and the results compared to a previously prepared standard curve and the dispense volume was interpolated.

Results

The time necessary for routine dispensing by the µFill was examined for various volumes at both the slowest and fastest dispense rates. The µFill can dispense 10 µl into each well of a 96-well microplate in less than 8 seconds and 5 µl into a 384-well microplate in approximately 10.5 seconds. This time includes the time required to move the plate carrier from the home position to the manifold at the beginning of the dispense cycle, as well as returning to the home position at the completion of the cycle. As one might imagine, with larger per-well volumes the time required to fill a plate increases. Dispense-times for deep-well plates were also measured at volumes of 500, 1000, and 2000 µl per well. As

demonstrated in Table 1, the time necessary for 300 µl to be dispensed into all 96 wells is 44.5 seconds at rate 1. However, at larger per-well volumes faster fluid rates are available which can cut the dispense time in as much as half. The degree of increase is dependent on the volume being dispensed, with larger volumes showing the greatest increase. Similar results were seen when 384-well plates were used. The standard model of the µFill Reagent Dispenser has five different dispense rates that affect the time required to fill a plate. The autoclavable model of the µFill uses a different syringe design that allows for faster dispense speeds. At the lowest volumes, the autoclavable µFill can dispense 10 µl into each well of a 96-well microplate approximately 0.5 seconds faster than the standard model (7.1 vs. 7.6 seconds) and for a 5 µl dispense into a 384-well microplate, it is approximately 0.7 seconds faster (Table 1). However, at larger volumes, the time savings from the autoclavable model become quite dramatic. For example, when dispensing 1000 µl into a deep-well 96-well microplate, the autoclavable model requires approximately 50 seconds to complete the task, while the standard model needs 135 seconds.

96-Well Dispense Times#					384-Well Dispense Times#				
	Standard Model		Autoclavable Model			Standard Model		Autoclavable Model	
Volume (µl)	Rate (µl/well/sec) 450	1000	Rate (µl/well/sec) 450	1000	Volume (µl)	Rate (µl/well/sec) 225	500	Rate (µl/well/sec) 225	500
10	7.6	-----	7.1	-----	5	10.4	-----	9.7	-----
20	8.1	-----	7.3	-----	10	12.7	-----	11.4	-----
30	9.8	-----	8.2	-----	20	17.9	-----	16.4	-----
40	11.0	-----	9.7	-----	30	22.8	-----	18.9	-----
50	12.1	-----	10.9	-----	40	28.1	-----	18.7	17.0
100	18.7	-----	11.7	10.7	50	33.6	-----	20.9	18.8
200	31.7	17.8	17.3	14.9	60	38.7	24.0	23.1	20.2
300	44.5	22.8	20.0	16.0	70	44.0	26.1	25.4	22.0
500	70.8	33.9	28.7	21.6	80	49.2	28.2	27.6	23.6
1000	135.6	59.6	50.1	35.9	90	54.3	30.1	29.8	25.3
2000	-----	115.4	97.5	68.8	100	59.5	31.9	32.0	27.0

Note that all times are in seconds

Table 1. Dispense times of Standard and Autoclavable models of the µFill. The dispense times indicated were measured with a hand-held stopwatch, starting from the point of initiating the dispense by pressing the “start” button on the keypad until the carrier had returned to the home position at the completion of the dispense cycle. The two rates used represent the slowest and fastest rate settings of 1 and 5, respectively. Note that rate 5 is not available for several of the smaller dispense volumes in either model.

The accuracy and precision of the µFill were determined using a number of different techniques. As demonstrated in Table 2, the µFill is accurate across the entire range of its volume settings for 96-well plates. When the minimum setting for 96-well plates (10 µl) was selected, the dispense-volume, determined gravimetrically, was found to deviate from expected by less than 3%. The deviation diminished with larger volumes to less than 1%. In all cases, the dispense volume was quite precise.

Dispense Accuracy of the μFill Dispenser into 96-Well Plates

Expected Volume (μl)	Calculated Volume (μl)#	% Deviation
10	10.3 ± 0.34	2.97
20	19.9 ± 0.57	0.34
30	29.4 ± 0.71	2.03
40	40.2 ± 1.09	0.50
50	49.9 ± 1.17	0.12
60	59.9 ± 1.23	0.20
70	69.9 ± 1.61	0.17
80	80.2 ± 0.91	0.27
90	90.1 ± 1.92	0.01
100	100.1 ± 2.03	0.12
150	150.3 ± 2.97	0.18
200	199.8 ± 3.92	0.12
250	250.3 ± 3.74	0.11
300	300.4 ± 3.17	0.14
500	497.9 ± 0.22	0.40
1000	997.6 ± 0.60	0.20

[#] Note that these data represent the mean and average of twelve determinations.

Table 2. Dispense accuracy into 96-Well plates. Individual microplates were weighed. After the μFill Dispenser had dispensed the indicated volume of fluid into each well of the plate, the plates were re-weighed. The volume of each well was calculated by dividing the change of weight by the number of wells (96) in a plate. Note that the data represent the average of 96 determinations. The percent deviation is the ratio of the difference between the calculated and expected values to the expected values.

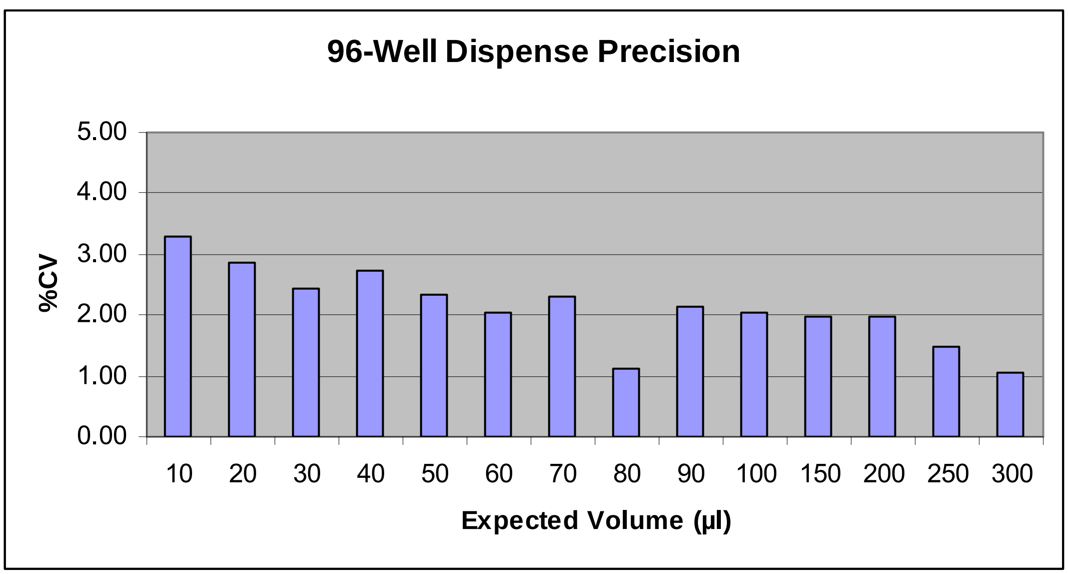


Figure 2. Dispense precision into 96-Well plates using the μFill 96-/384-Well Reagent Dispenser at various dispense volumes. Each of the indicated volumes of deionized water was dispensed to microplates using the μFill. For each plate of 96 wells, an average per well dispense-volume was calculated as previously described. Note that each data bar represents the %CV for the average of the 96 wells.

As demonstrated in Figure 2, the coefficient of variance (%CV) was found to be less than 4% at 10 µl per well. The %CV also decreased with larger volumes to less than 2% at volumes above 100 µl per well. When the dispense accuracy into 384-well microplates was examined, similar results were found. When dispensing 5 µl into each well of a 384-well plate, the average deviation was calculated to be approximately 1% (Table 3). Again, as the per-well volume was increased the deviation from expected diminished to less than 1%. The precision when dispensing into 384-well plates was also quite good, with CVs being less than 4% at the lowest volume setting (Figure 3). Because the µFill enables the selection of different fluid dispense-rates, the accuracy of pipetting different volumes at different rates was examined.

Dispense Accuracy of the µFill Dispenser into 384-Well Plates

Expected Volume (µl)	Calculated Volume (µl)[&]	% Deviation
5	4.95± 0.30	1.01
10	9.76 ± 0.22	2.43
20	20.22 ± 0.62	1.12
30	29.87 ± 0.79	0.45
40	39.96 ± 0.69	0.11
50	50.07 ± 0.86	0.13
60	60.00 ± 0.95	0.01
70	69.98 ± 0.99	0.02
80	79.92 ± 1.05	0.11
90	89.84 ± 0.85	0.18
100	99.88 ± 1.18	0.12

[&] Note that these data represent the mean and average of 384 determinations.

Table 3. Dispense accuracy into 384-Well plates. Nunc flat-bottomed 384-well microplates were weighed. After the µFill Dispenser had dispensed the indicated volume of fluid into each well of the 384-well plate, the plates were re-weighed. The volume of each well was calculated by dividing the change of weight caused by the number of wells (384) in a plate. The percent deviation is the ratio of the difference between the calculated and expected values to the expected values. Each data point represents the mean and standard deviation 384-wells.

Figure 3. demonstrates the dispense precision into 384-well plates. The increase in absolute values most likely reflects the natural variation of the meniscus of the solution in microplate wells. Note that in order to obtain adequate coverage of the wells with solution, it was necessary to add deionized water to all wells such that the final volume was 100 µl.

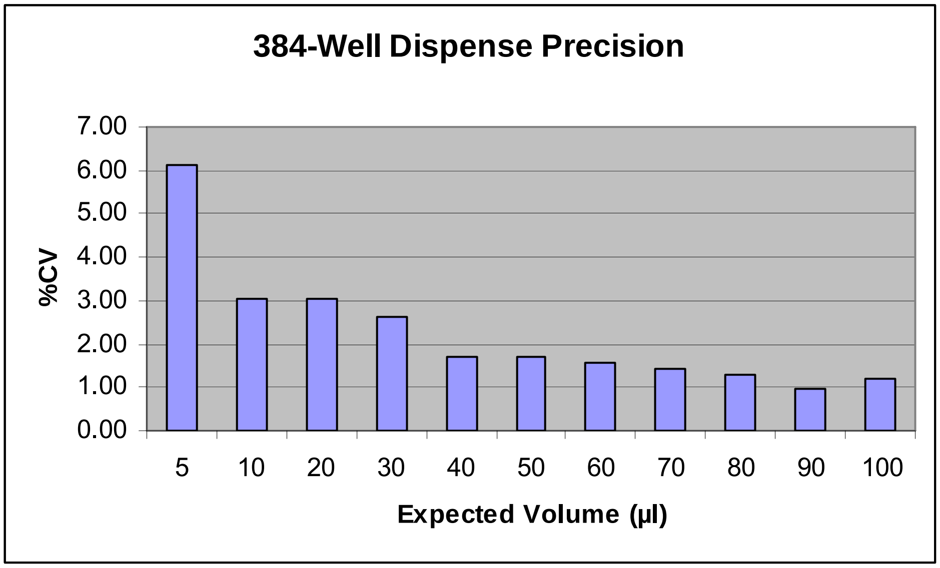
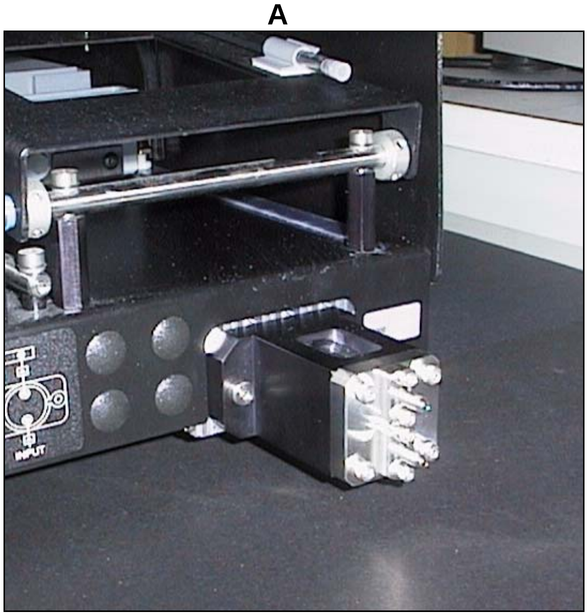


Figure 3. Dispense precision into 384-Well plates using the µFill 96-/384-Well Reagent Dispenser at various dispense volumes. Each of the indicated volumes of deionized water was dispensed to eight 384-well microplates using the µFill. For each plate, an average per well dispense-volume was calculated gravimetrically. Note that each data bar represents the %CV for the average of the eight plates.

The autoclavable version of the µFill, besides providing an easy means for sterility, also provides an easy access for cleaning purposes. Removal of the syringe piston and barrel is easily accomplished. As demonstrated in Figure 4A, the external syringe is located at the rear of the µFill, protruding approximately 3 inches. The barrel is attached with two hex-screws that are removed with the provided hex-wrench (Figure 4B). Once the barrel has been removed, the piston is exposed and can be removed after loosening a setscrew, using the same wrench (Figure 4C). The barrel, piston, tubing, check-valves, and dispense manifold can then be sterilized by autoclaving. One can expect that these parts will provide accurate liquid-dispenses after a minimum of 50 autoclave-sterilization cycles. In addition, extra components of the fluid-path assembly can be purchased, allowing for sterilization of one set while another set is in use.

A



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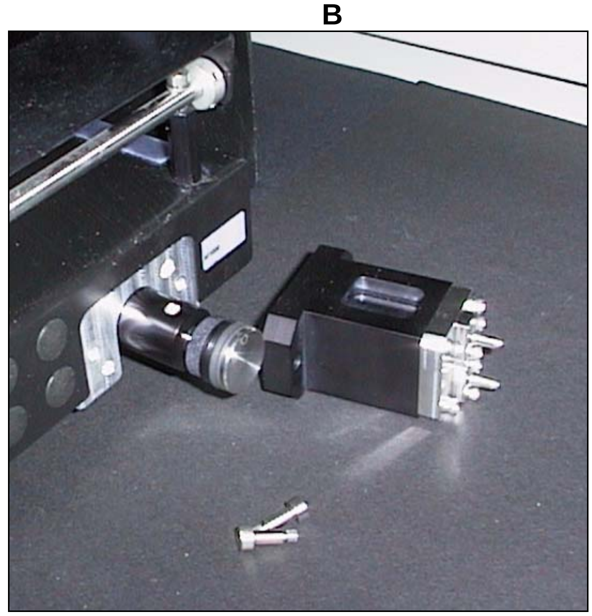

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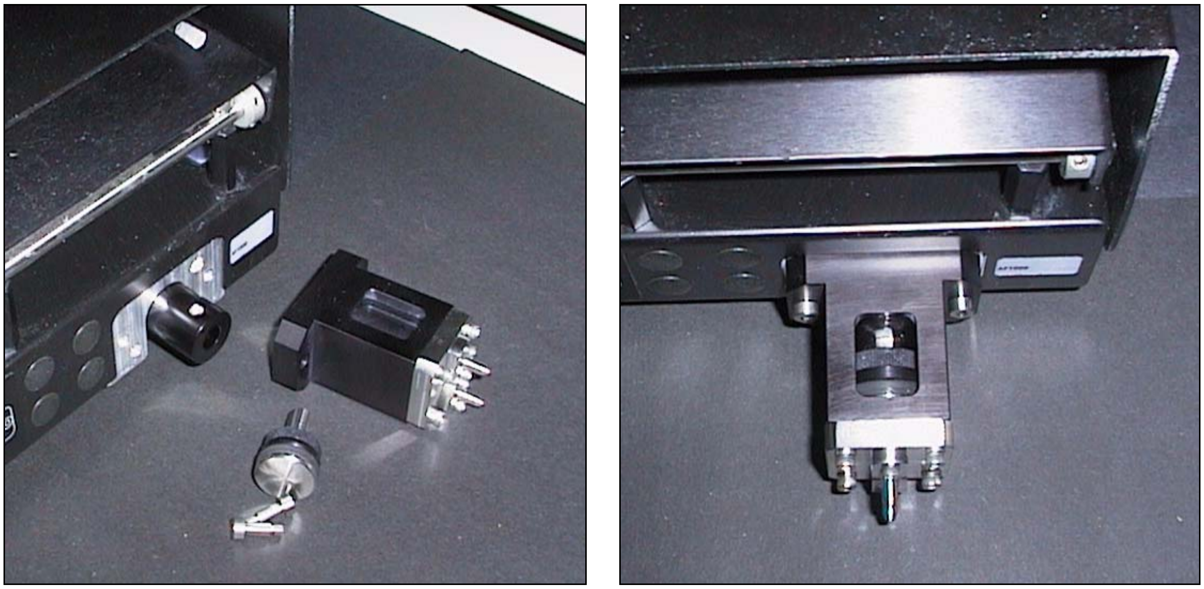


Figure 4. External syringe unit located at the rear of the autoclavable model of the µFill Reagent Dispenser. Picture A depicts the rear of the autoclavable µFill Reagent dispenser with the external syringe unit attached. Note that one of the two retaining screws can be seen at the base of the syringe assembly. Picture B shows the syringe barrel removed with the syringe piston still attached. The setscrew that retains the piston can be seen as a light-colored dot at the top of the housing. Picture C shows the syringe assembly with barrel and piston removed prior to autoclaving, while Picture D depicts the reassembled syringe unit from the top. Note that the tubing port fittings can be seen protruding out the back.

Sephadex Bead Dispense Accuracy into 96-Well Plates					
Gravimetric Method			Absorbance Method		
Expected (µl/well)	Volume (µl)	%CV	Expected (µl/well)	Volume (µl)	%CV
100	100.39	0.399	100	106.63	5.32
200	201.72	0.228	200	202.00	1.38

Table 4. Dispense accuracy of the µFill for Sephadex into 96-Well Microplates. After dispensing the indicated volume of Sephadex Superfine G-50 beads into 1 x 8 strips of a 96-well microplate using an autoclavable model of a µFill Reagent Dispenser, the per-well dispense volumes were calculated using either gravimetric or absorbance methods. Assuming a specific gravity of 1 g/ml, the average per-well volume was calculated for the change in weight of microplate strips after dispensing. The resultant absorbance measurements at 405 nm caused by light scattering was used to estimate dispense volumes by interpolation of a previously prepared standard curve.

Figure 5. Microplate strip with Dispensed Sephadex beads. Using the autoclavable model of the µFill, 300 µl of Sephadex Superfine G-50 beads in suspension were dispensed to each well of several 1- x 8-well strips in a frame. After dispensing the beads, a strip was removed and photographed. Note the well-to-well uniformity of bead suspension.

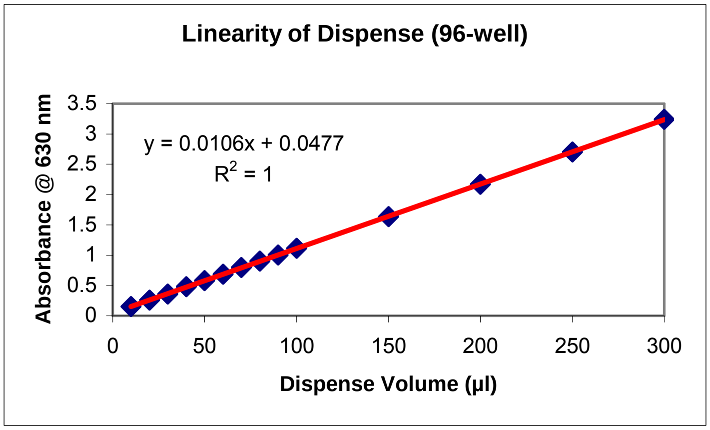
Sephadex Bead Dispense Accuracy into 384-Well Plates					
Gravimetric Method			Absorbance Method		
Expected (µl/well)	Volume (µl)	%CV	Expected (µl/well)	Volume (µl)	%CV
50	50.20	-----	50	51.63	1.95
75	75.51	-----	75	77.13	1.85
100	100.75	-----	100	96.50	2.13
120	119.60	1.41	120	123.71	3.03

Table 5. Dispense accuracy of the µFill into 384-Well Microplates. After dispensing the indicated volume of Sephadex Superfine G-50 beads into Nunc 384-well microplates using an autoclavable model of a µFill Reagent Dispenser, the per-well dispense volumes were calculated using either gravimetric or absorbance methods. Assuming that the suspension has a specific gravity of 1 g/ml, the average per-well volume was calculated for the change in weight of microplate after dispensing. The resultant absorbance measurements at 405 nm caused by light scattering was used to estimate dispense volumes by interpolation of a previously prepared standard curve.

The µFill can be used to dispense colloidal suspensions, such as Sephadex beads. Fine particles that remain in suspension can be dispensed into 96- and 384-well microplates with a high degree of accuracy and precision. As demonstrated in Table 4, using a gravimetric method to determine volume, when volumes of 100 or 200 µl are used, the autoclavable model of the µFill is quite accurate, with determined dispense-volumes of 100.39 and 201.72 µl, respectively. This represents a deviation of less than 1% for either volume. The precision in either case was very high, as the %CV was less than 0.5%. Interestingly, it was found that the light absorbance caused by the Sephadex beads could be used to determine the volume dispensed into microplate wells. While any wavelength could be used, the 405 nm was the most repeatable. This wavelength is in the region where the lamp output of the µQuant Microplate Spectrophotometer (BioTek Instruments) is the greatest, which suggests that different microplate readers may require different wavelengths. When the absorbance at 405 nm of wells filled with either 100 µl or 200 µl of bead suspensions were interpolated from a previously prepared standard curve, determined volumes of 106.6 µl and 202 µl were returned, respectively. These data are in close agreement with the gravimetric determinations. The slight differences between the two methods can most likely be attributed

to the increased variability of the absorbance method. This is corroborated by the precision data, which show higher CVs. An example of the well-to-well uniformity of the bead suspension is depicted in Figure 5.

The µFill can also be used to fill 384-well plates with Sephadex beads. As demonstrated in Table 5, volumes of Sephadex bead suspensions ranging from 50 µl to 120 µl can be dispensed accurately and precisely. Regardless of the plate matrix, when dispensing bead suspensions, it was necessary to agitate the suspension reservoir in order to prevent the suspension from settling was empirically determined. The setting for a magnetic stirrer that maintained the suspension. In addition, if there was any sort of delay between plates when dispensing with the system, priming was required. Priming served to re-suspend the beads in the lines, while failure to prime the dispenser resulted in much higher CVs.



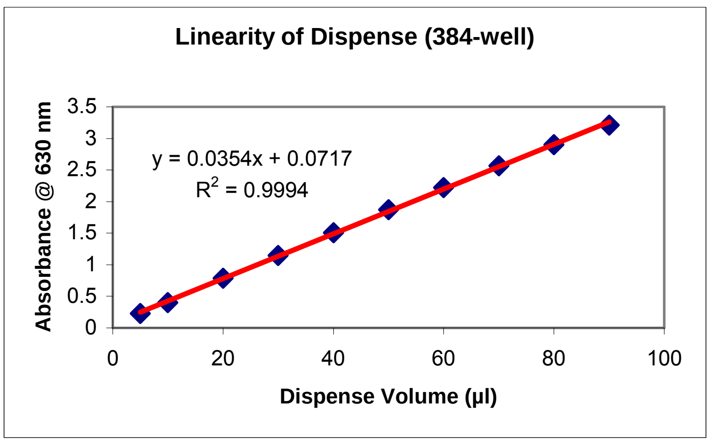


Figure 6. Linearity of Dispense using the µFill Reagent Dispenser. Several different dispense protocols were run on separate plates and the mean absorbance plotted. After dispensing, deionized water was added to the wells of the microplate such that all of the wells contained either 100 µl of solution for 384-well plates or 300 µl of solution for 96-well plates. The absorbance at 630 nm was then determined for each well using a Synergy HT Microplate Spectrophotometer. The mean absorbance at each volume (diamonds) was plotted along with the linear regression (line).

Figure 6 demonstrates the linearity of dispense of the µFill reagent dispenser. Several individual dispense routines were run in succession, each with a different volume setting. Volumes of dye solution ranging from 5 to 90 µl were dispensed into 384-well microplates. Likewise, similar experiments were performed in 96-well plates with volumes ranging from 10 to 300 µl. After dispensing the dye solution, deionized water was added to all wells such that each well contained 100 µl or 300 µl of solution for 384- and 96-well plates respectively and the absorbance for each well of the microplate was determined. When the mean absorbance

at each volume was plotted against the volume dispensed, a linear relationship was observed. When linear regression analysis of the data was performed, the coefficient of regression (r^2) was calculated to be greater than 0.999. The resolution of dispense is demonstrated in Figure 7. When dispense routines in increments of 1-µl are linked together and the resultant absorbance values plotted, the mean value for each volume is very linear. The coefficient of regression for a dye solution was calculated to be greater than 0.999. Note that while minimum and maximum values at each volume overlap, the two parallel the same trend line as the mean.

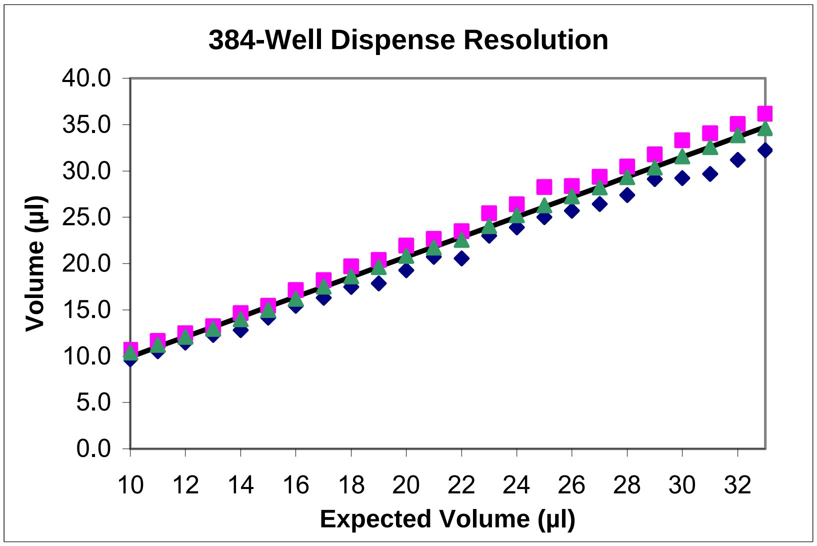


Figure 7. Dispense volume resolution of the µFill Reagent Dispenser. Using the "link" function of the µFill, several different dispense protocols were linked together such that various volumes of yellow dye solution were dispensed to strips of a Nunc 384-well plate in 1-µl increments. After dispensing, the absorbance at 450 nm (630 nm reference) was then determined for each well and the values interpolated against a previously prepared standard curve. Data points representing the minimum (diamonds), maximum (squares), and mean (triangles) values at each volume were plotted along with the linear regression (line).

Discussion

These data indicate that the µFill Reagent Dispenser can be used to dispense either large or small volumes precisely and accurately. The µFill routinely exceeds the accuracy and precision specifications for 20 µl and 80 µl dispense volumes. BioTek's 6-point in-house calibration procedure provides a high degree of accuracy at any volume setting. In addition, because the µFill uses a syringe pump, accuracy is maintained without repeated calibrations by the end-user. The µFill is accurate at any valid dispense rate, allowing the end-user to decide the appropriate rate based on their application. The µFill's design is such that it can accommodate 96- and 384-well plates without changing the manifold. Deep-well microplates can be accommodated by removal of the plate holder from the carrier, while dispensing into plate types with intermediate heights can be accomplished via software control of the manifold dispense height. Using different optional 8-channel manifolds also allows the µFill to dispense into 24-well and 96-well half area microplates. Multiple dispense routines can be linked together, allowing multiple per well volumes, rows to be skipped, different dispense rates, all within the same plate.

There are several priming features available with the µFill. The priming trough has a fitting that allows for a drain tube to be connected. Besides the prevention of overflow from the trough by removal of primed reagent, the reagent can be recollected and precious reagent

can be saved. Built-in software features include an AutoPrime™ function that allows for periodic priming of the reagent dispenser during periods of inactivity. Selection of the "New Buffer Prime" provides for a sufficient priming volume to purge the fluid path of one reagent and replace it with another.

The autoclavable model of the µFill offers several advantages over the standard model. The most obvious, of course, is sterility. The entire fluid path can be removed and steam-sterilized using a conventional autoclave. Chemical sterilization with agents such as alcohol is no longer necessary. The ability to access the entire fluid path also allows for easier cleaning. Suspensions, such as Sephadex beads, if left in the syringe will settle over time.

The easy removal of the fluid components allows the user to manually clean the components. Additionally, the switching between aqueous buffers and organic solvents may require that the syringe be removed and cleaned and dried between solvents, as polar and non-polar solvents often are immiscible. The autoclavable version of the µFill provides increased organic solvent resistance. The material from which several of the components of the µFill have been changed in order to withstand the heat and pressure associated with autoclaving has the added benefit of being resistant to many organic solvents. Using the autoclavable model in conjunction with optional organic-solvent-resistant check valves allows DMSO, acetonitrile, and butanol to be dispensed using the µFill. The diminutive size of the µFill allows it to be easily used in a chemical fume hood. The externally located syringe pump provides for quieter operation, and improvements in the syringe aspiration profiles have allowed for quicker refilling of the syringe, resulting in faster per-plate dispense times. The ability to accurately dispense fine colloidal suspensions such as Sephadex beads has great utility. While not tested in this treatise, there are several other types of fine-bead suspensions that could also be utilized. The most obvious are magnetic bead particles. These particles have a number of different surface properties that allow them to bind substances specifically, yet at the same time have ferrite properties that allow them to be captured by magnetic forces. As with Sephadex, these beads would need to be kept agitated in order for them to be accurately dispensed. In addition, cell suspensions can be dispensed using the µFill. While all cell lines are different, the dispense rate for each tube of the µFill can be adjusted to as low as 225 µl/sec, which would allow many different types to be dispensed into 96- and 384-well plates.

The small footprint of the dispenser allows for easy use in biosafety cabinets, as well as incorporation into automated systems. The µFill is based on proven washer technology that ensures a high degree of accuracy and precision for long periods of time. The manifold and tubing are easily accessible for cleaning or replacement.

Paul Held Ph.D., Applications Scientist
Jason Greene, Product Manager

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