Tech Note

Automated Dispensing of Scintillation Cocktail using the µFill™ Microplate Reagent Dispenser

Introduction

The use of automated dispensers dedicated to microplates allows for large numbers of samples to receive reagents very rapidly. In general, these reagents are non-viscous aqueous solutions and with proper care and instrument maintenance, easily performed. However, a significant number of techniques require the addition of viscous and/or non-aqueous solutions. For example, compound libraries are often reconstituted with dimethyl sulfoxide (DMSO) rather than water due to concerns regarding aqueous solubility. Polymerase Chain Reactions (PCR) are often overlaid with mineral oil to prevent evaporative volume and concentration changes in the reaction during cycling. Many enzymatic reactions contain significant amounts of glycerol to improve performance or to prevent ice crystal formation. Radioactive assays, e.g. RIA, $[H]^{3-}$ thymidine incorporation, $[P]^{32}$-kinase reactions, etc. require the addition of scintillation cocktails in order to convert radioactive decay into light photons, which can subsequently be measured by a luminescence or scintillation reader. While every fluid mixture presents its own set of unique problems, there are a number of common traits shared by many viscous solutions, the most notable of which is the resistance of the fluid to movement or flow. In this tech note we describe the use of the µFill™ Microplate Reagent Dispenser to dispense scintillation cocktail to microplates, as an example of viscous fluid dispensing.

Figure 1. µFill™ Microplate Reagent Dispenser

Table 1. Differences Between Expected and Observed Dispense Volumes.

Expected Volume (µl)	Observed Volume (µl)*	Difference (µl)	Percentage of Expected
50	35.99	14.01	71.99
100	82.36	17.64	82.36
150	133.37	16.63	88.91
200	182.52	17.48	88.91

* Data represents the mean of 12 determinations

Materials and Methods

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Microscint 40 scintillation cocktail was obtained from Perkin-Elmer, Life and Analytical Sciences (Boston, MA). 1 x 8 well clear Polysorb Microplate strips were purchased from Nunc. A µFill™ Reagent Dispenser (BioTek Instruments, Winooski, VT), configured with an 8-channel manifold, was programmed to dispense various amounts of Microscint 40 scintillation cocktail to 12 (1 x 8) microplate strips. A flow rate of 1 (150 µl/well/sec), which is the slowest setting, was used along with the default settings for dispense height and offset. An aspiration delay of 2500 milliseconds was enabled with a prime before dispense.

The aspiration delay can be enabled from the keypad by accessing the UTIL menu, then selecting "TESTS", then pressing the "SHIFT" and Hidden Key #2 (space between the main menu and Previous Screen buttons). (See Figure 2.) Next select "SYR", then "DELAY". Enter the delay in milliseconds (2500). *Note: BioTek found a 2500 millisecond aspiration delay to be appropriate for Microscint 40 scintillation cocktail. However, users should first validate performance before altering the aspiration delay from the default of 0 milliseconds with other (i.e. aqueous) solutions.*

Figure 2. Programming Keypad for the µFill Reagent Dispenser. Dispense programs, maintenance routines and instrument utilities can be accessed and programmed directly from the instrument keypad. Under the UTIL menu, select TESTS. To access the aspiration delay command, press the Shift and Hidden key #2 (area between the Main Menu and Previous Screen buttons) simultaneously. Select SYR then DELAY to define the length of aspiration delay in milliseconds.

Gravimetric determinations were performed by weighing individual 1 x 8 strips using a Sartorius A 120S analytical balance. A total of 12 strips for each volume were tested. The dispense volume accuracy into 96-well microplates was determined by weighing each empty strip before and after dispensing the Microscint 40 cocktail with the µFill. The average weight per well was calculated by dividing the difference between initial and final weights (Delta) by the total number of wells (8). Using the specific gravity of water (0.96 g/ml), a conversion from weight to volume was then made. The mean from the 12 determinations was determined, as well as the standard deviation and %CV for each volume.

Results

The µFill™ Reagent Dispenser was used to dispense various volumes of Microscint 40 scintillation cocktail into the wells of microplate strips. As demonstrated in Figure 3, there was a direct correlation between the intended dispense volume and the actual volume dispensed by the µFill. In each volume tested, the actual amount was slightly less then the intended volume.

Interestingly, the difference between the expected volume and the observed volumes was consistant regardless of the volume (Table 1). This suggests that the discrepancy is due to resistance to flow within the fluid path of the dispenser rather than inaccuracy of the syringe pump. The elasticity of the fluid-line tubing in conjunction with the resistance to flow of the viscous solution most likely results in a certain amount of transient stretching of the tubing, which results in the dispense volumes. The dispense precision, as measured by the coefficient of variation (%CV), is very good at all volumes tested. The %CV with a 50 µl-dispense was found to be 7%, with large dispense volumes having even lower variations (Figure 4).

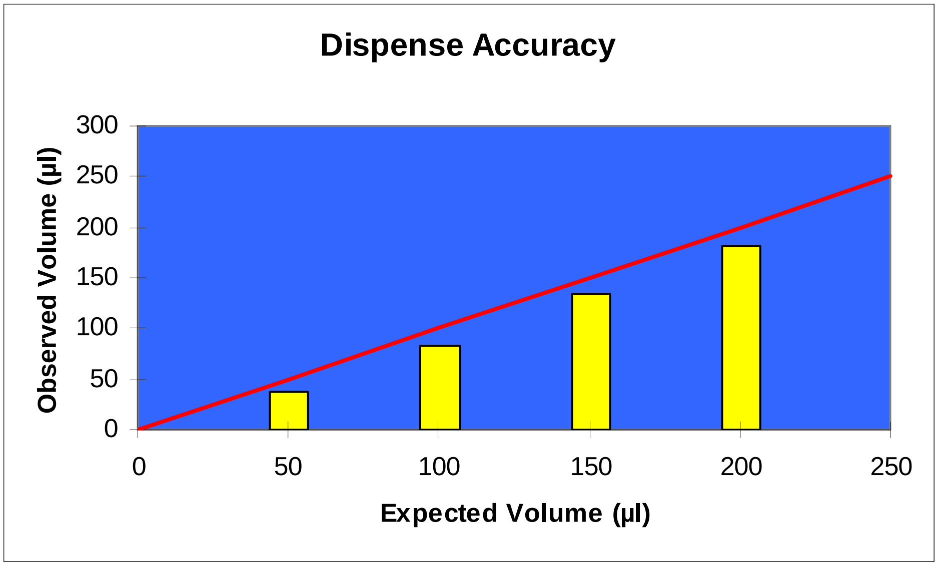

Figure 3. Dispense Accuracy with Microscint 40 Scintillation Cocktail. The indicated volumes of Microscint 40 cocktail were dispensed into 1 x 8 microplate strips and the strips individually weighed with the dispense volume calculated from the specific gravity. Afterward, the average per well dispense volume was calculated by measuring the change from before and after dispensing and dividing by the number of wells. The mean of 12 strips at each volume was then averaged and plotted. The red line indicates the theoretical expected volume.

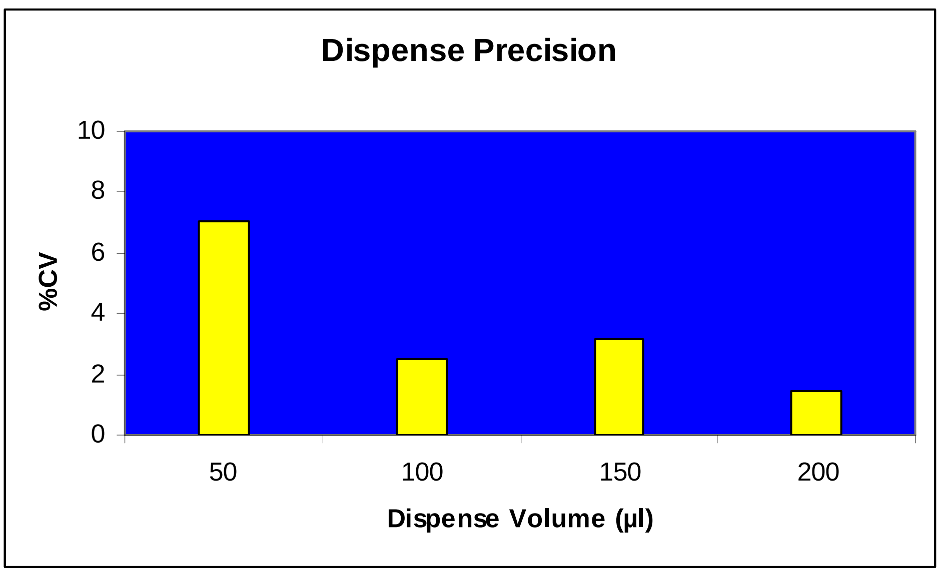

Figure 4. Dispense Precision with Microscint 40 Scintillation Cocktail. Various volumes of Microscint 40 cocktail were dispensed into 1 x 8 strips in a microplate frame using the µFill Reagent Dispenser. Using gravimetric measurements, the mean dispense volumes of the wells for each 1 x 8 strip were calculated

along with the standard deviation and %CV. The %CV was plotted as a function of the intended dispense volume.

Discussion

Two key features were found to be extremely important when dispensing viscous fluids such as scintillation cocktail. The aspiration delay software feature allowed for a sufficient delay for fluid movement with solutions such as Microscint 40 cocktail. This delay provided time for the viscous fluid to move through the tubing and out the dispense tubes. Without the delay considerable amounts of dripping and poor precision occurred as a result of the carrier moving to the next strip before the fluid had finished flowing. (Note that the µFill basecode software versions 1.21 or greater are required for the aspiration delay capability.) In addition, the manifold plugs need to be the most recent BioTek design. With the new design (AF1000 models – P/N 8050707, AF1000A models – P/N 7110553) the plugs are more solid in structure and incorporate a double o-ring seal rather than the original rubber flanged plug (Figure 5). Use of the original plug design often resulted in blowouts and subsequent loss of dispense fluid.

Viscous solution dispensing entails a certain number of challenges. Viscous fluids by their nature have a degree of reluctance toward flow. The resultant backpressure in the tubing and manifold generated by the syringe requires the use of the newly designed manifold plugs, which incorporate a double o-ring type seal. This design grips the manifold much better preventing “blowouts” which can occur if the backpressure exceeds the grip of the plugs. While this is the standard plug design on newly manufactured µFill dispensers, the older µFills utilized the older design. Note that access to the openings that these removable plugs fill is necessary for proper cleaning of the manifold.

Figure 5. Original and New Manifold Plugs. The original style rubber manifold plugs (left) have been replaced with a more rigid design (right) that incorporates two O-rings to make a liquid tight seal.

When dispensing viscous solutions into 96-well microplates it is recommended that the 8-channel manifold (P/N 7140515S) be used rather than the 16-channel that normally is provided. The resistance of the tubing surface impedes the flow of viscous fluids. The less surface area of the 8-channel manifold is more amenable to fluid movement than the 16-channel manifold.

In addition to 96- and 384-well microplates, the µFill can also dispense solutions to 24-well plates when configured with another 8-channel manifold (P/N 7140518S) specifically designed to accommodate these microplates. This manifold has the dispense tubes grouped in pairs to accommodate the 4 x 6 matrix of a 24-well plate. The use of 8 tubes allows for sufficient fluid flow, while maintaining the dispense accuracy and precision. The user need only identify the manifold in the instrument UTIL menu.

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