Technical Note: 07002

Saving Reagents via Process Automation

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Key Words
- Thermo Scientific Matrix PlateMate
- Automated Pipetting
- Saving Reagents

Abstract

The Thermo Scientific Matrix® PlateMate® 2x3 is a six-position versatile automated liquid handling platform. The six-position, flexible deck configuration accommodates microplates, reservoirs, wash stations, vacuum manifolds and additional accessories. This experiment was conducted to test the capabilities of the Matrix PlateMate 2x3 on sample conservation and accuracy of dispensing. The Matrix PlateMate 2x3 was used in conjunction with an 8-channel column serial dilution magazine to distribute 10 µl aliquots of liquid to a 384-well plate. Sample conservation was affected by the use of a serial dilution magazine and a 96-well plate as a reagent reservoir. The 96-well plate was used as an alternative to a reagent reservoir and allowed for minimal residual liquid. Most automation reagent reservoirs require a minimum of 20 ml of liquid to cover the ridged bottom and to allow for proper aspiration.

Introduction

Recent trends in pharmaceutical development indicate the use of lower quantities of expensive reagents to reduce cost and increase throughput. In routine experimentation, researchers often use reagent reservoirs to dispense the stock solution into several microplates. Oftentimes, this generates residual reagent left in the reservoir after the liquid transfer. With the increase in cost of individual assay runs, scientists must investigate ways to prevent unnecessary wastage of expensive reagents while simultaneously maintaining a high level of accuracy and precision. Automated platforms such as the Matrix PlateMate 2x3 and the Thermo Scientific Matrix PlateMate Plus eliminate the human error that can occur during manual pipetting and the variability encountered by different users. In addition to automated equipment, consumables such as reservoirs also play an important role in achieving proper aspiration/dispension. For example, Thermo Scientific Matrix Automation Reservoirs are coated to create a hydrophilic surface with grooves at the bottom that reduce dead volume required. These grooves allow the liquid to spread evenly to occupy the entire bottom surface of the reservoir. Although such alternatives exist, it is still possible to further reduce the wastage of reagents. In this study, we investigated the possibility of using a 96-well plate and the Matrix PlateMate 2x3 with the ControlMate software program in lieu of a reagent reservoir. Since the 96-well plate uses less volume than the reagent reservoir, the reagent cost can be significantly minimized. Furthermore, Thermo Scientific Matrix automated liquid handlers provide the flexibility to use different types of plates and reservoirs on the stage which will enable the user to use the appropriate plate as a reagent reservoir.

Materials:

1. 1% Tartrazine Dye Solution
2. Thermo Scientific Matrix PlateMate 2x3 (Item no. 801-10001)
3. 300 µl Thermo Scientific Matrix D.A.R.T.s® Tips (Item no. 5516)
4. Thermo Scientific Matrix 96-Well Polypropylene Microplate, Clear, U Bottom, Sterile (Item no. 4918)
5. Thermo Scientific Matrix 384-Well Polystyrene Microplate, Clear, Flat Bottom (Item no. 4310)
6. Thermo Scientific Matrix Serial Dilution Magazine (Item no. 501-30050)
7. Thermo Scientific Matrix WellMate® (Item no. 201-10001)
8. Plate Shaker
9. Tecan Genios Reader
10. Centrifuge
11. ControlMate Software Version Matrix PlateMate 2x3 Pre-Release 1.1.1
12. Thermo Scientific Matrix 1250 µl Filtered Pipette Tips, Sterile (Item no. 8045)
13. Thermo Scientific Matrix Single Channel Electronic Pipette, 15-1250 µl (Item no. 1024)

Methods:

The Matrix PlateMate 2x3 was programmed via ControlMate Pre-Release Version 1.1.1.

1. Using a Matrix WellMate, 90 µl of diH20 was added to each plate*
2. 300 µl of reagent was aspirated from the first column of a 96-well plate
3. 10 µl was dispensed into each well of the 384-well plate in the following pattern:

Example Pattern of Reagent Dispensing

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	5	6	9	10	13	14	17	18	21	22
B	3	4	7	8	11	12	15	16	19	20	23	24

☐ represents 1 well

4. Using the Matrix PlateMate 2x3, the remaining reagent was dispensed back to the 96-well plate (to preserve the greatest amount of reagent)
5. The reagent wells were refilled with a manual pipette
6. 10 µl was dispensed to the second half of the plate in a similar manner as that of the first half (see 3 above)

Example Pattern of Reagent Dispensing

	13	14	15	16	17	18	19	20	21	22	23	24
A	25	26	29	30	33	34	37	38	41	42	45	46
B	27	28	31	32	35	36	39	40	43	44	47	48

☐ represents 1 well

7. The plates were then shaken on a plate shaker at setting 6 for 5 min.
8. Plate was centrifuged at 1750 rpm for 1 min.
9. Plates were scanned using a Tecan Genios Reader set to 405 nm at 5 flashes

* In the case of dry dispense, 90 µl of diH20 was added after running the plates through the ControlMate sequence, and in the case of wet dispense, the water was added to the plates before being run through the ControlMate sequence

Results:

The performance of the Matrix PlateMate 2x3 was tested to deliver microliter volumes of liquid, using a 96-channel Air Displacement pipetting head and a single column of tips. The Matrix PlateMate 2x3 was fitted with an 8-channel serial dilution magazine using 300 µl Matrix D.A.R.T.s tips. Both wet and dry dispenses were performed as described in the methods and tested for accuracy and precision (Tables 1 & 2). The results indicate that an accuracy and precision of greater than 97% could be achieved with this procedure.

Table 1:

Dispensing 10 µl of 1% Tartrazine dye solution into 90 µl H_2O

Wet	Absorbance	CV	Volume (µl)
Plate 1	1.9609	3.51%	10.20
Plate 2	1.9801	2.04%	10.30
Plate 3	1.9785	2.12%	10.29
Average	1.9732	2.56%	10.26

Table 2:

Dispensing 10 µl of 1% Tartrazine dye solution into an empty plate

Dry	Absorbance	CV	Volume (µl)
Plate 1	1.9237	5.70%	10.01
Plate 2	1.9173	1.33%	9.98
Plate 3	1.9167	1.36%	9.97
Average	1.9193	2.80%	9.99

Note: The higher CV% in Plate 1 of each dispense type in Table 1 and 2 above is a result of the tips being dry; These values would be improved by implementing a pre-wet. Evaporation within the tip can cause a significant loss of sample before delivery and pre-wetting increases the humidity within the tip. Plate 1 dispense mimics a pre-wet step that would improve CV%.

Wet dispense was tested in triplicate as shown in Table 1. The average absorbance value of these plates shows low variability across the plate according to the Coefficients of Variance (CV). Also, the error on these plates is low. Dry dispense was also tested in triplicate (see Table 2). These plates show an average CV of less than 3% which indicates low variability across the plate. The error on these plates is also very low, as seen in Table 2.

Conclusion:

Utilizing the 96-well plate instead of a reagent reservoir allows the user to dispense 10 µl of reagent into each well of a 384-well plate with less than 4 ml of reagent. The Matrix PlateMate 2x3 gives users the flexibility to make use of different vessel types as a reservoir. Standard reagent reservoirs require at least 20 ml of dead volume in addition to the sample volume that may be used. With this procedure and equipment conformation, it is possible to complete dispenses using only 4 ml of reagent. Based on the results demonstrated above, the Matrix PlateMate 2x3 is able to dispense liquids with a low CV and high accuracy as well as saving reagents needed for the experiment.

Acknowledgements: Thanks to Tal Murthy, Ph.D., Kiara Williams and Dan Shaffer. This work was performed at the Thermo Fisher Scientific facility in Hudson, New Hampshire, USA.

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