

# A Wash Protocol to Determine and Eliminate Liquid Carry-Over Using the Thermo Scientific Matrix® PlateMate® 2x2 with Stainless Steel Syringes

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## Key Words

- Thermo Scientific Matrix PlateMate
- Liquid Carry-Over
- Wash Cycle

## Abstract

Liquid carry-over in the syringes of a positive displacement pipetting head can introduce cross-contamination to samples and interfere with the precision and accuracy of dispenses. Determining the amount of liquid carry-over and a method by which to eliminate this contamination is important when the syringes are used in multiple aspirate and dispense cycles. In this study, the Thermo Scientific Matrix PlateMate 2x2, a compact and versatile automated pipetting station, is equipped with a 384-channel positive displacement pipetting head with stainless steel syringes. The purpose of this study is to determine the percentage of liquid, specifically 90% DMSO with 10% dye, carried-over in the syringes and the number of wash steps necessary to produce negligible carry-over. It was determined that after five wash cycles the average absorbance of the liquid in the plate was less than that of a background plate. However, after visual inspection of the plate, a sixth wash cycle was necessary to remove contamination from all channels of the pipetting head. The data from this study showed that a total of six wash cycles are required to completely remove residual liquid contamination. In order to conserve valuable time and Matrix PlateMate deck space, it was determined that a 384-channel standard height tip wash station can be used to perform the six consecutive wash cycles to achieve elimination of liquid contamination in syringes between dispenses.

## Introduction

The Matrix PlateMate 2x2 is a 96- or 384-channel automated pipetting workstation configurable with any of eight interchangeable air-displacement or positive displacement pipetting heads (see Figure 1). The four-position work deck can accommodate a variety of accessories including tip washing stations, vacuum manifolds, piercing manifolds and barcode readers. The instrument is equipped with two peristaltic pumps which can be used to fill reagent reservoirs and tip washing stations and the non-contact liquid level detection feature of these pumps ensures that reagent reservoirs are automatically replenished when designated without overfilling.

The Matrix PlateMate 2x2 is compatible with an unlimited amount of laboratory applications: row- or column-based serial dilutions, cell assays, sterile pipetting,



Figure 1: Matrix PlateMate 2x2.

filter plate vacuum assays, pipetting resins, beads, caustic solvents and high vapor liquids, as well as others, which can be conformed to specific laboratory functions via ControlMate software. ControlMate software, a Windows®-based program with a user-friendly graphical interface, gives users programming control and flexibility. This software is equipped with a grouping function that allows programs to loop in order to perform tasks such as serial dilutions. For security purposes, ControlMate allows the user to place an edit and run lock through password protection.

Experiments which require interchangeable pipetting of different reagents or pipetting for multiple series using the same pipetting head, have the possibility of carrying over residual liquid contamination. Therefore, it is very important to develop a method to incorporate a series of wash cycles to ensure that the amount of liquid carry-over from one pipetting sequence to the next is negligible. DMSO, because of its wide use as a sample solvent, was prepared in solution with tartrazine dye to determine liquid carry-over. In this study, a protocol for the number of wash cycles necessary for minimal carry-over is determined and further adapted for efficiency and space-saving with a 384-channel tip wash station.

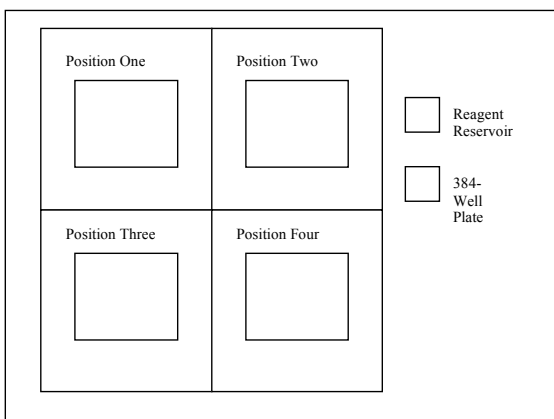
**Materials:**

1. Thermo Scientific Matrix PlateMate 2x2 (Item no. 301-10005)
2. Thermo Scientific Matrix Positive Displacement Pipetting Head, 384-Channel, 0.1-50 µl (Item no. 501-102890)
3. ControlMate Software Version 1.3.38
4. Thermo Scientific Matrix WellMate® (Item no. 201-10001)
5. Thermo Scientific Matrix Standard Height Tip Wash Station, 384-Channel (Item no. 30002)
6. Tecan Plate Reader
7. Centrifuge
8. Plate Shaker
9. DMSO Solution: 90% DMSO with 10% Tartrazine Dye Solution
10. Thermo Scientific Matrix 384-Well Polystyrene Microplate, Clear, Flat Bottom (Item no. 4310)
11. Thermo Scientific Matrix Disposable Automation Reservoir, 384-Channel (Item no.1064-05-7)
12. Distilled Water

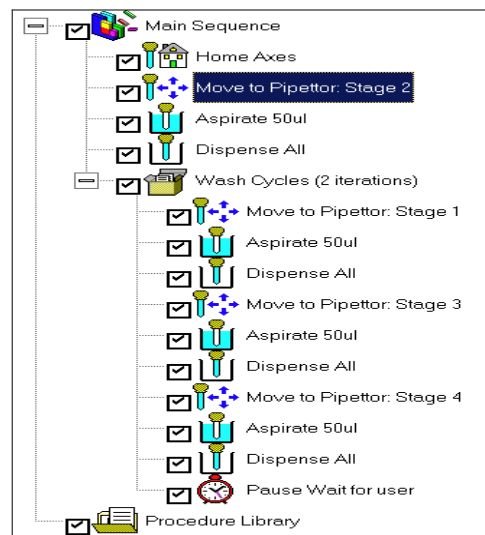
**Methods:**

**Procedure Used to Determine Percentage of Liquid Carry-Over**

1. Stock of 90% DMSO with 10% tartrazine dye solution was prepared
2. Using the Matrix WellMate, 384-well plates were filled with 100 µl of distilled water
3. Matrix PlateMate deck set-up (see Figure 2):
  - a. Reagent reservoir was filled with prepared DMSO/dye solution and placed into position two on the Matrix PlateMate deck
  - b. Filled 384-well plates were placed in positions one, three and four on the deck
4. Using ControlMate software, the following program was followed for determination of liquid carry-over (see Figure 3):



**Figure 2: Matrix PlateMate deck configuration for tip wash protocol.**

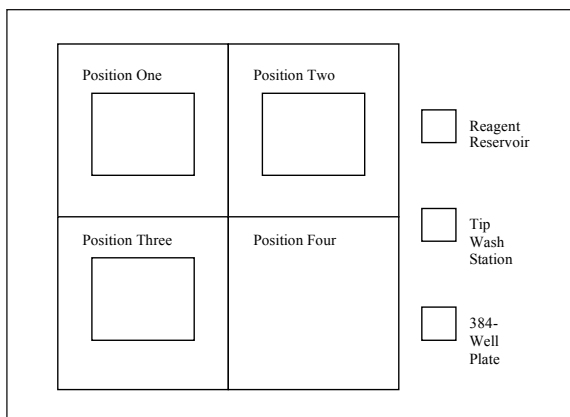


**Figure 3: ControlMate protocol used to run tip wash in six different plates.**

- a. 50 µl of prepared DMSO/dye solution was aspirated from the reservoir and then dispensed directly back to the reservoir
  - b. The 384-channel positive displacement pipetting head moved to the first position and aspirated 50 µl from the plate and dispensed it directly back into the plate
  - c. The previous step was repeated for the third and fourth positions
  - d. After finishing the first wash cycle iteration, the program incorporated a pause. At the pause, the user removed the three plates on the deck and three fresh plates filled with distilled water were placed on the deck. The user clicked “Continue” and the pipetting head moved to each plate, aspirated 50 µl and dispensed back to the plate
5. All of the plates were shaken for 5 min. on a plate shaker and then spun down at 1750 rpm for one min.
  6. The plates were read using a Tecan Genios Reader set to 412 nm at 5 flashes
  7. A plate containing only distilled water was also read in order to produce a background measurement
  8. Averages, standard deviations and the coefficient of variance were calculated and recorded for each plate

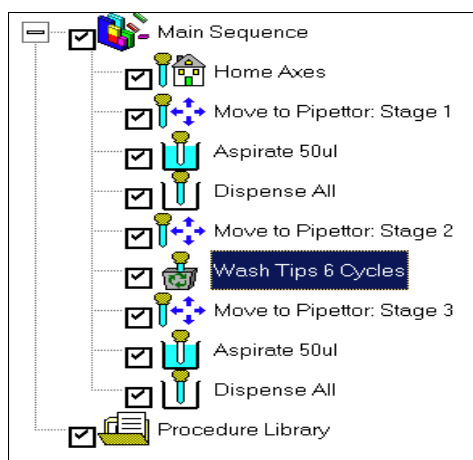
**Procedure Using the Matrix 384-Channel Tip Wash Station**

1. Matrix PlateMate deck set-up (see Figure 4):
  - a. A standard height, 384-channel tip wash station was placed in position two of the Matrix PlateMate deck and attached to the peristaltic pump In/Out plugs on the deck
  - b. Reagent reservoir was filled with prepared DMSO/dye solution and placed into position one on the Matrix PlateMate deck
  - c. Filled 384-well plate was placed in position three of the deck



**Figure 4: Matrix PlateMate deck configuration for tip wash station protocol.**

2. Using ControlMate software, the following program was used to determine elimination of liquid carry-over via use of a wash station (see Figure 5):
  - a. First, a “zero” plate, a plate obtained by a run with no wash cycle steps, was produced by aspirating and dispensing 50 µl of prepared DMSO solution into the reagent reservoir and then moving to a fresh filled 384-well plate and aspirating and dispensing 50 µl water
  - b. Wash station was primed using distilled water
  - c. 50 µl of prepared DMSO/dye solution was aspirated from the reservoir in position one and then dispensed directly back to the reservoir
  - d. The pipetting head moved to the tip wash station on deck position two and performed one to six wash cycles, aspirating 50 µl from the tip wash chimneys and then dispensing the waste into the waste bath
  - e. The pipetting head moved to deck position three and aspirated 50 µl from the 384-well plate and dispensed 50 µl directly back into the plate
3. This cycle was repeated in triplicate for each number of wash cycle iterations, each time placing a fresh 384-well plate in position three



**Figure 5: ControlMate protocol used to operate the 384-well tip wash station and also aspirate and dispense into a fresh filled 384-well plate, determining if liquid carry over has been eliminated.**

4. All of the plates were shaken for 5 min. on a plate shaker and then spun down at 1750 rpm for one min.
5. The plates were read using a Tecan reader set to 412 nm at 5 flashes
6. A plate containing only distilled water was also read in order to produce a background measurement
7. Averages, standard deviations and the coefficient of variance were calculated and recorded for each plate

## Results

In order to determine the amount of dye carried over from each wash, the average absorbance of the plates were compared to the first wash plate. It would have been unrealistic to compare the first wash plate to a plate containing only the prepared DMSO/dye solution because the absorbance of a plate containing only DMSO/dye with 10% dye was too high to be read accurately by the Tecan reader. Therefore, all of the subsequent plates were compared to the first wash plate (see Table 1).

**Table 1:**  
**Calculated Carry-Over Between Syringe Washes**

Wash #	Average Absorbance	Background Subtracted	% Carried over from Wash #1
Background	0.0349		
1	1.5621	1.5272	
2	0.2225	0.1876	12.3
3	0.0523	0.0174	1.14
4	0.0349	0.0000	0.00
5	0.0308	-0.0041	Average Absorbance Less Than Background
6	0.0305	-0.0044	Average Absorbance Less Than Background

After the second wash cycle, 12.3% of the dye was carried over to the second plate and after the third wash cycle, only 1.14% of the dye had remained in the syringes. The procedure was carried out for a fourth, fifth and sixth wash cycle because, after visualization of the plates (even though the average absorbance of the plate was equal to or less than that of the background plate), there were still individual wells where slight amounts of dye could be seen. Inspection of the plate after the fifth wash showed very faint traces of dye in a few individual wells and upon completion of the sixth wash, there was no visible dye in the plate.

To make this protocol more efficient and applicable in laboratories, the data from Table 1, determining the number of necessary wash cycles, was adapted to a wash station. Using a Matrix standard height tip wash station, similar end point data was obtained while taking less time and using minimal deck space (see Table 2).

**Table 2:****Calculated Carry-Over and Length of Each Protocol Using the Tip Wash Station**

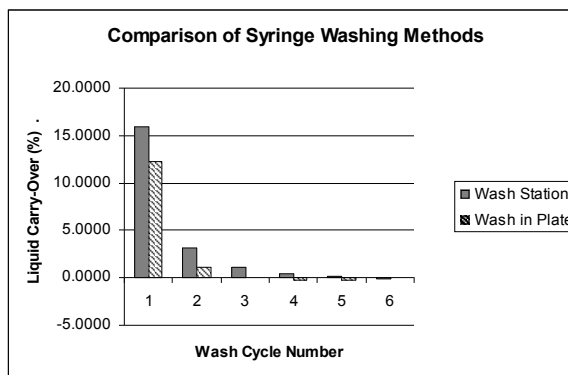
Wash Cycle	Average Absorbance	Background Subtracted	% Carried Over From Wash #1
Background	0.0349		
0	1.5089	1.47	
1	0.2702	0.2353	15.9
2	0.0805	0.0456	3.09
3	0.0506	0.0157	1.06
4	0.0416	0.0067	0.45
5	0.0363	0.0014	0.09
6	0.0341	-0.0008	Average Absorbance Less Than Background

The first wash plate in Table 1 was produced before any washes were performed. In order to be comparable to the first procedure, a “zero” plate was made during the wash station protocol before any wash cycles occurred. All of the plates produced during the wash station protocol were compared to the “zero” plate. After one wash cycle, 15.9% of the dye remained in the syringes and after two cycles 3.09% of the dye remained. The procedure was carried out for six wash cycles. After washes four and five, there were still faint traces of dye seen in a few individual wells, as in the first wash protocol. After six washes, the average absorbance was less than the background and there was no visible dye in the plate.

The two protocols, wash in plate and wash station, were compared (see Figure 6). The first protocol, wash in plate, showed less carry-over between washes and the average absorbance of the plates dropped below the background in fewer wash cycles than the second protocol, wash station. Wash cycle one, plate one, from the second protocol correlates with plate two from the first protocol, therefore the second protocol contained seven plates because of the necessity for the “zero” plate.

**Conclusion**

This procedure was performed to determine the percentage of dye which was carried over between washes of the 384-channel, positive displacement pipetting head syringes as well as to determine the number of washes necessary to eliminate this carry-over. It was determined by the first protocol, that in order to eliminate any carry-over of dye, it is necessary for the syringes to be washed at least five to six times, dependent on the solution. The exact wash protocol of this experiment would be impractical for laboratory use, because of the space taken up on the Matrix PlateMate deck. However, the information collected here was applied to using a wash station.



**Figure 6:** In this comparison of methods by which the syringes were washed, the bars display percent carry-over from syringes washed in the tip wash station and diagonal-lined bars, carry-over from syringes washed in individual plates.

Using a 384-channel standard height tip wash station to wash the syringes six times successfully and comparably eliminated carry-over in the syringes. The wash station protocol required one more wash cycle than the wash in plate protocol and the plates took more wash cycles for the absorbance to drop below the background plate. This is likely because, in the wash station protocol, the liquid for each wash is being pipetted from the same chimney, where the tip wash in plate protocol moved to a fresh plate each time. However, the space and time saving benefits of using a wash station greatly outweigh any inconveniences of the necessity of the extra wash cycle required to achieve the same end result. The amount of washes necessary to attain negligible carry-over is dependent on the substance being pipetted and, while it is expected that this protocol will work with a wide range of common laboratory buffers and reagents, further studies would be necessary to determine the exact number of washes needed for less common substances.

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