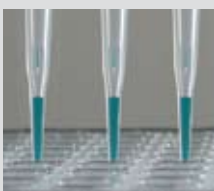


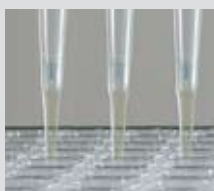
Guide for using Thermo Scientific PocketTips D.A.R.T.s on the Thermo Scientific Matrix PlateMate 2x3 , PlateMate Plus, and Hydra DT

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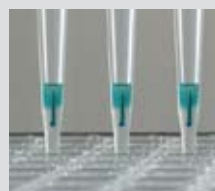
- Key Words
- Automated Liquid Handling
 - PocketTips
 - D.A.R.T.s
 - Compound Screening
 - PlateMate Plus
 - PlateMate 2x3
 - Low Volume Pipetting
 - Nanoliter Liquid Transfers
 - DMSO transfers



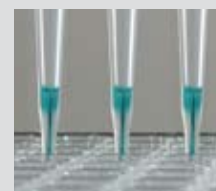
Step 1: Source Plate: Sample aspirated and pocket fills to capacity (50 nl, 100 nl or 250 nl)
Step 2: Source Plate: Sample aspirated and dispensed back to vessel and pocket remains full



Step 3: Wash Plate: The portion of the tip below the pocket is rinsed with water to eliminate carryover



Step 4: Destination Plate: Liquid in destination plate is aspirated to pocket level in the tip



Step 5: Destination Plate: Sample from the pocket and liquid from the destination plate are dispensed back to the destination plate

Introduction

Thermo Scientific PocketTip D.A.R.T.s (Disposable Automation Research Tips) enable customers to transfer between 50-250 nl of sample volume using Thermo Scientific Matrix PlateMate and Matrix Hydra DT liquid handling instruments. These low volume transfers allow the reduction of reagent, labware, and sample costs while improving data quality by eliminating intermediate pipetting steps and providing soft in-tip mixing. The PocketTip D.A.R.T.s include an internally molded capillary pocket that holds the fixed compound in DMSO nanoliter volume and are designed to form a surface seal with the current selection of fixed and interchangeable 96- and 384- format pipetting heads.

Included in this user guide are stepwise instructions for set up of the PocketTip D.A.R.T.s tips on compatible Matrix automated liquid handling platforms. Instructions with and without tip wash station are included.

Materials

1. PlateMate Plus, PlateMate 2x3, or Hydra DT
2. Microtiter plates
3. PocketTip D.A.R.T.s 96- or 384- format
4. Compounds dissolved in DMSO (Dimethyl Sulfoxide) source plate
5. Aqueous buffer or reagents (destination plate)

Methods

The following procedure is optimized for labware specifications and source/ destination liquid volumes. Individual adjustments may be required for optimal PocketTip D.A.R.T.s use with other manufacturer's labware.

The following method illustrates a generic PocketTip D.A.R.T.s pipetting method created to work for 96- and 384- format 30 µl Extended Length PocketTip D.A.R.T.s tips with 50 nl, 100 nl, or 250 nl pocket. Sample ControlMate PocketTip DARTs programs are available for download from www.controlmate.net and www.thermo.com/matrix.

Program the PlateMate Plus or PlateMate 2x3 with a Generic PocketTips Routine (Tip Washer on the Deck)

After setting up the deck with the desired source and destination labware, create a method to use the PocketTip D.A.R.T.s. The above method follows the methodology for dispensing 50 nl, 100 nl, or 250 nl volumes and includes the subroutines listed on the next page.

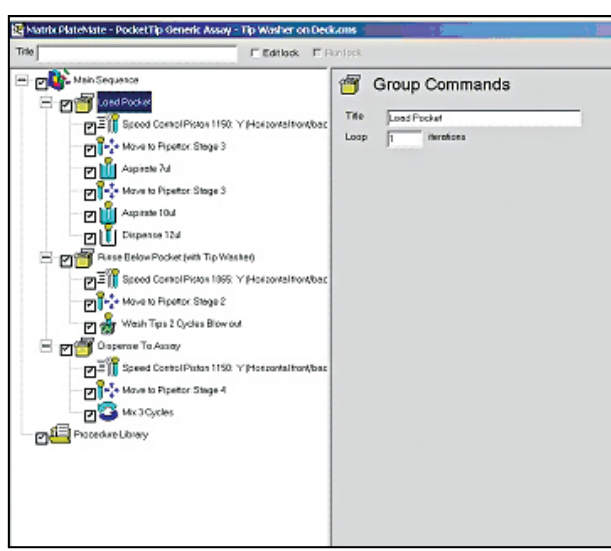


Figure 1: A ControlMate program, including the 3 subroutines is shown. Individual commands are shown in the following figures.

Subroutines for this procedure include:

- I. Load Pocket
- II. Rinse Below the Pocket
- III. Dispense to Assay

Subroutines

- I. Load Pocket
- II. Rinse Below the Pocket
- III. Dispense to Assay

I.1 Speed

Note: For best transfer results, a slow piston speed is recommended for DMSO transfer for pocket fill and empty of DMSO.

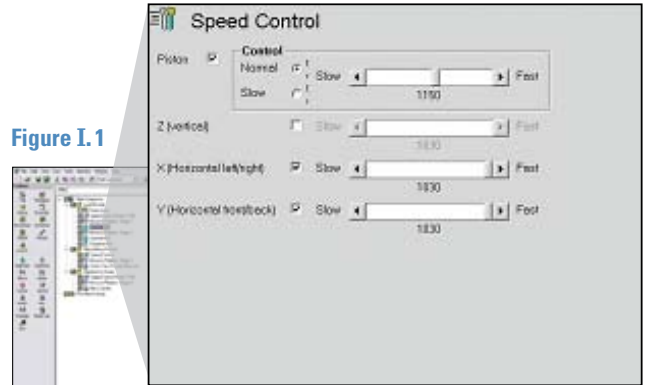


Figure I.1

I.2 Move to Pipettor: Stage 3

Note: Position 3 contains the DMSO source plate.

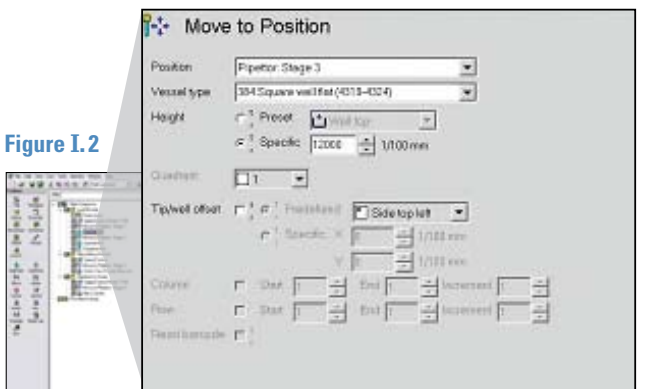


Figure I.2

I.3 Aspirate Airgap: 7 µl

Note: Aspirate an airgap to be consumed throughout the first 2 subroutines, load pocket and rinse below the Pocket.

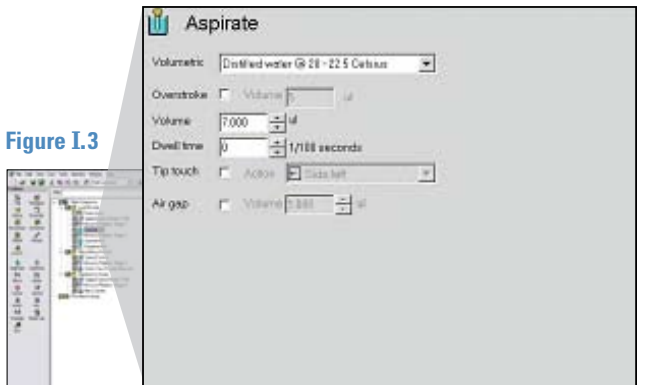


Figure I.3

I.4 Aspirate to Fill Pocket with DMSO compound; Move to Pipettor: Stage 3

Note: Position 3 contains the DMSO source plate. The tips should be set to 0.5-1.0 mm from the bottom of the well. Ensure a minimum volume of 1 µl of DMSO is available.

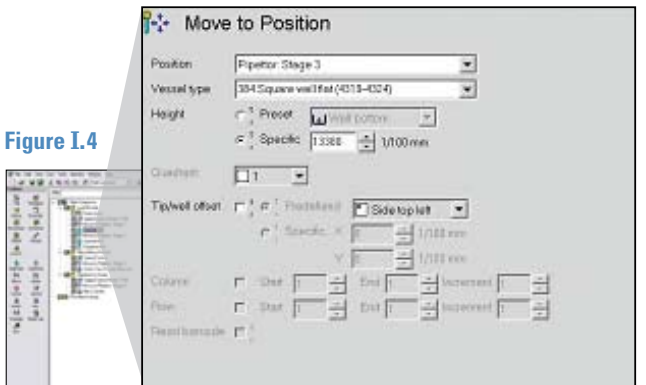


Figure I.4

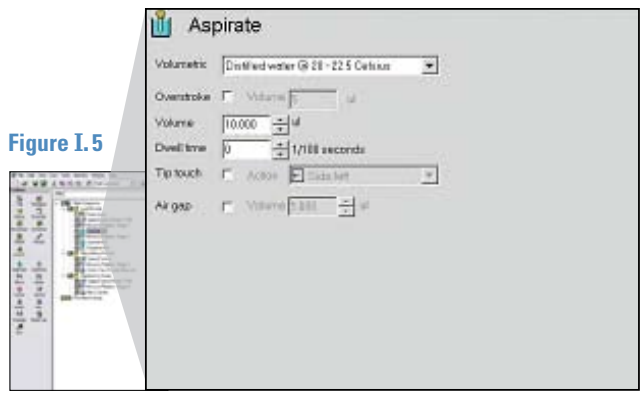


Figure I.5

I.5 Aspirate 10 µl

Note: Aspirate the DMSO just beyond the top of the pocket to fill with fixed volume.

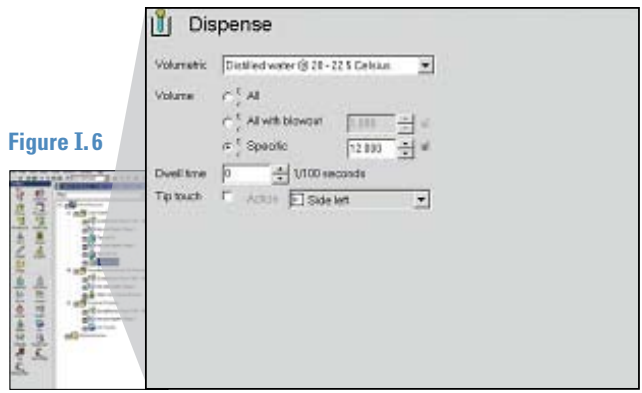


Figure I.6

I.6 Dispense 12 µl

Note: Dispense the remaining DMSO back to the source plate. An additional 2 µl to evacuate the tip has been added to the dispense volume. The pocket remains filled. Verify that no more than 0.25 µl of DMSO remains in the tip aperture (waste).

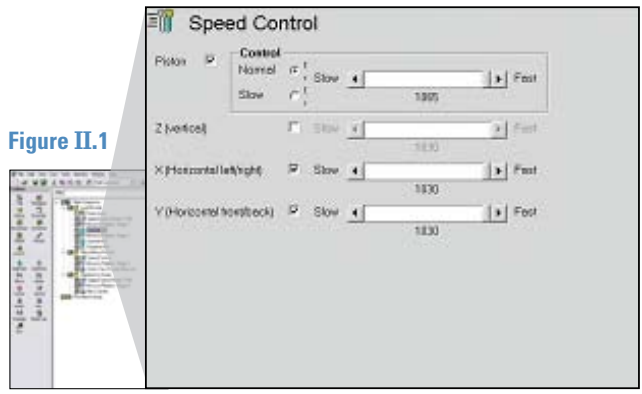


Figure II.1

Subroutines

- I. Load Pocket
- II. Rinse Below the Pocket
- III. Dispense to Assay

II.1 Speed

Note: Increasing the piston speed during this subroutine will decrease method cycle time.

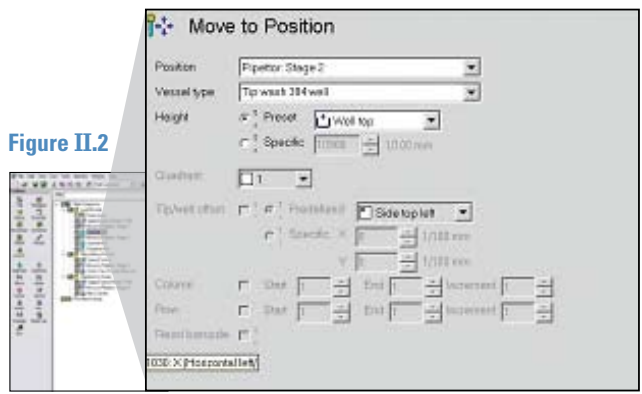


Figure II.2

II.2 Move to Pipettor: Stage 2, tip washer position

Note: This position contains the tip washer. Prior to running the method, the tip washer should be primed with distilled water.

II.3 Wash Tips 2 Cycles 3 μ l with Blowout

Note: This step rinses the tip below the pocket but does not contact the pocket with the water. A blowout volume of 5 μ l will adequately empty the tip after washing. Note, the airgap aspirated in step 1C has been completely depleted by steps I.6 and II.3. Caution: Do not apply a blowout volume above 5 μ l unless the airgap volume in step I.3 is increased by the same amount.

- Subroutines**
- I. Load Pocket
 - II. Rinse Below the Pocket
 - III. Dispense to Assay

III.1 Speed

Note: Reducing the speed to the speed used in Step I.A allows the buffer and reagents time to dwell over the pocket offering adequate transfer efficiency and in-tip mixing.

III.2 Move to Pipettor: Stage 4

Note: Position 4 contains the assay plate with aqueous buffer and reagents in each well. 10 μ l per well is sufficient for flat bottom 384- well plates and 5 μ l is sufficient for round bottom 384- well labware. Tips should be located in the well such that 2ul can be aspirated.

III.3 Mix 3 cycles of 9 μ l

Note: The mix cycles will aspirate the buffer until it contacts the pocket opening. The aqueous buffer's surface tension will contact the DMSO and empty the pocket. Three mix cycles are satisfactory when running an assay due to average incubation times following compound addition. Increase the mix cycles to 10 if you are performing % CV testing with fluorescein to ensure adequate mixing in the well before reading, shown in Figure III.4 (next page).

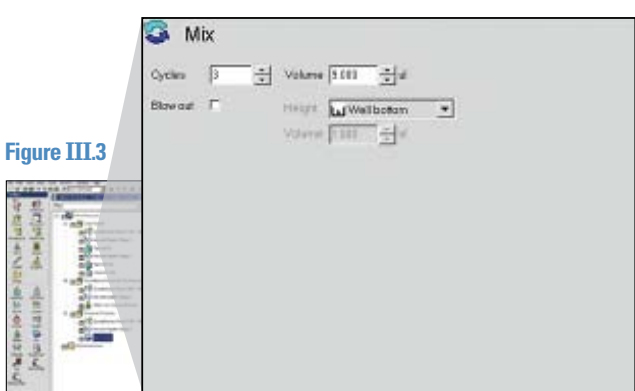
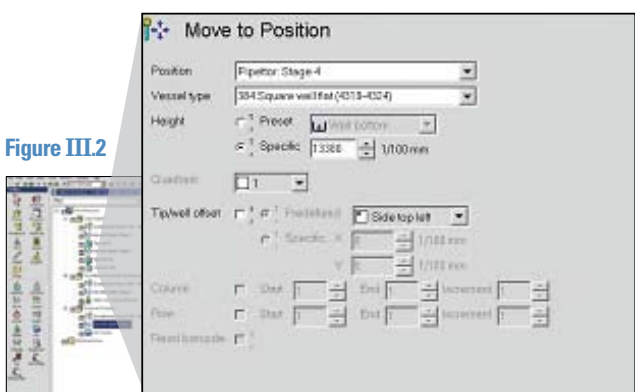
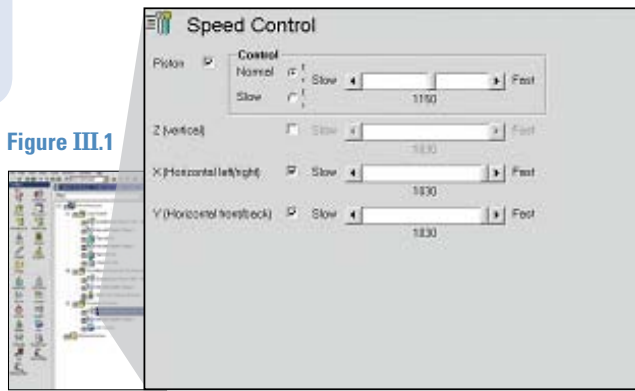


Figure II.3

Figure III.1

Figure III.2

Figure III.3



Figure III.4

OPTIONS:

Program the PlateMate Plus or PlateMate 2x3 with a Generic PocketTips Routine (no tip washer present)

If using a PlateMate Plus or PlateMate 2x3 without a tip washer on the deck, the following modification to subroutine 2 will be necessary. Subroutine 1 and 3 remain the same as above. This method will work with 50, 100 and 250 nl PocketTip D.A.R.T.s. The method contains (3) subroutines:

Subroutines

- I. Load Pocket (same as above)
- II.A Rinse Below the Pocket
- III. Dispense to Assay (same as above)

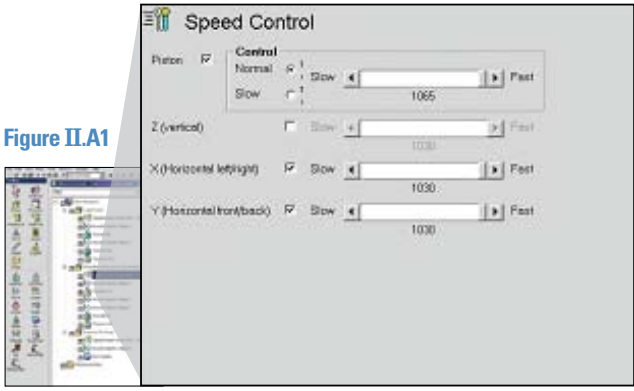


Figure II.A1

II.A1 Speed

Note: Increasing the piston speed during this subroutine will decrease method cycle time.

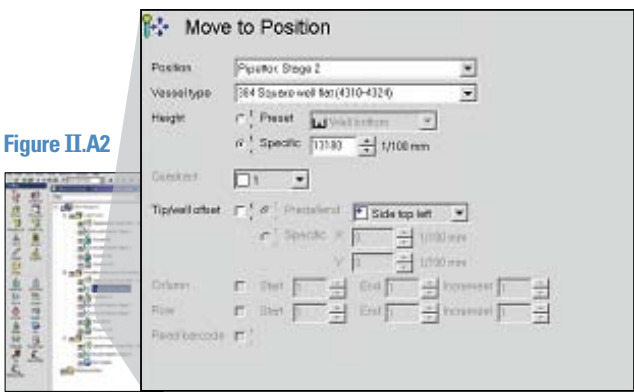


Figure II.A2

II.A2 Move to Pipettor: Stage 2

Note: This position contains a reservoir or microplate containing water. The reservoir or plate should be 75-100% filled.

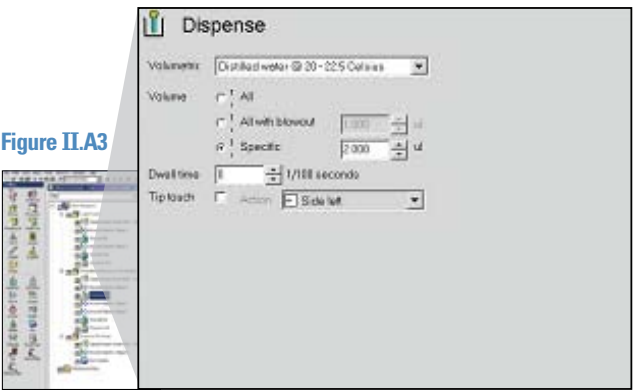


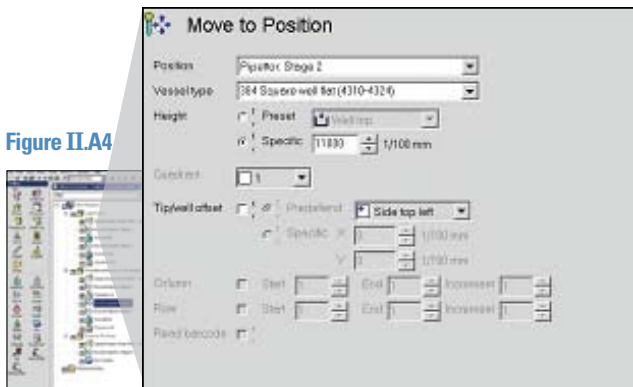
Figure II.A3

II.A3 Dispense 2 µl

Note: This step evacuates any DMSO meniscus remaining in the tip aperture. Be sure that the tips are submerged in the water to a level deeper than they were in the source plate. This will ensure an adequate external tip rinse.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

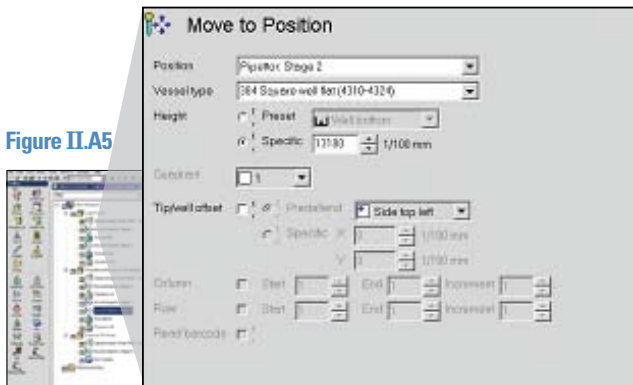
Figure II.A4



II.A4 Move to Pipettor: Stage 2

Note: This movement breaks off the air bubble that was created in step II.A3.

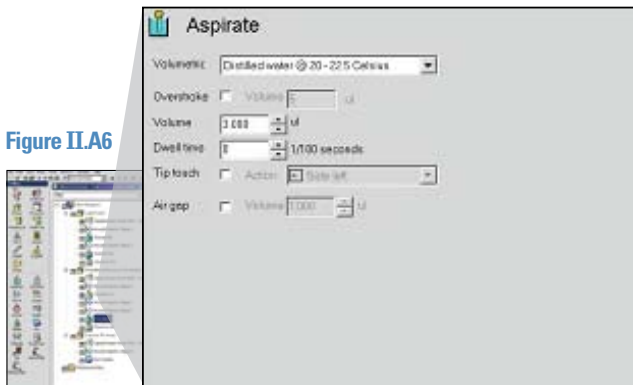
Figure II.A5



II.A5 Move to Pipettor: Stage 2

Note: This movement positions the tips back into the reservoir or microplate containing water.

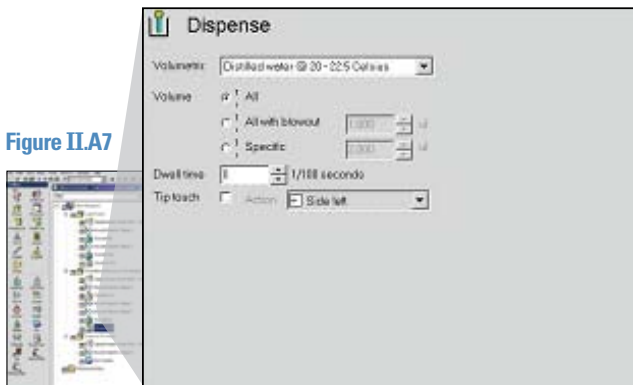
Figure II.A6



II.A6 Aspirate 3 μ l

Note: 3 μ l is sufficiently below the pocket level and will provide an adequate rinse.

Figure II.A7



II.A7 Dispense All

Note: A Dispense All step will use up the remaining 5 μ l airgap from step I.C. Caution: do not apply a larger blowout volume than 5 μ l unless the airgap volume in the step I.C is increased by the same amount.

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