Performing Serial Dilutions with the Thermo Scientific Versette Automated Liquid Handler

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Introduction

The Thermo Scientific™ Versette™ is a compact automated liquid handler for a wide range of research laboratories suitable for plate replication, plate reformatting and serial dilutions. The six-position, flexible deck configuration accommodates standard and deep-well plates in both 96- and 384-well plate formats, reagent reservoirs and 96- and 384-SBS formatted Thermo Scientific™ Matrix™ Sample Storage tube racks. The Versette also includes several accessories such as a tip wash station, and with the purchase of a pump, a non-contact reservoir fill is included free of charge.

The intuitive graphical Thermo Scientific™ ControlMate™ software allows users to quickly set up and run serial dilutions. For optimal serial dilution, it is important to aspirate at the bottom of the well, dispense at the top of the well, and mix efficiently.

The Versette is compatible with a total of three user-interchangeable air displacement pipetting heads available to use for serial dilutions. These pipetting heads utilize Thermo Scientific™ D.A.R.T.’s™ (Disposable Automation Research Tips) and cover volume ranges of 0.5 μl–30 μl or 5.0 μl–300 μl in a 96-channel configuration and 1.0 μl–100 μl in a 384-channel configuration.

For this experiment, the Versette was used with a 1x8 column formatted serial dilution magazine to perform a 1:1.5 serial dilution across a 96-well plate. 300 μl standard D.A.R.T.’s were loaded in the first column of the serial dilution magazine.
Why Serial Dilute?
Serial dilution of a cell line can be used to estimate cell counts for different stock solutions, or to analyze cell culture concentration series against an array or panel of a compound.

Serial dilution with small molecules can be used to test activity/reactivity or synthesis to determine the extinction coefficient, LC50, or a simple titration.

However, serial dilutions of reagents, molecule compounds or cells can be tedious and time-consuming in many laboratory procedures. In addition, using manual methods can increase the risk of injuries and stress factors associated with pipetting ergonomics.

Methods
Serial dilutions are simple to program with the Versette.

The master plate set up to create the serial dilution is shown in Figure 3:

<table>
<thead>
<tr>
<th>Column 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 = 300µl Red dye</td>
</tr>
<tr>
<td>B1 = 200µl Red dye / 100µl Yellow dye</td>
</tr>
<tr>
<td>C1 = 100µl Red dye / 200µl Yellow dye</td>
</tr>
<tr>
<td>D1 = 300µl Yellow dye</td>
</tr>
<tr>
<td>E1 = 150µl Yellow dye / 150µl Green dye</td>
</tr>
<tr>
<td>F1 = 300µl Green</td>
</tr>
<tr>
<td>G1 = 150µl Green dye / 150µl Blue dye</td>
</tr>
<tr>
<td>H1 = 300µl Blue dye</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Columns 2–12</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl diH2O</td>
</tr>
</tbody>
</table>

Materials
1. Thermo Scientific™ Multidrop™ Combi (#5840300)
2. Deionized distilled water
3. Versette (#650-INSTR) with ControlMate software
4. 96 Channel 300 µl pipetting head (#650-06-96300)
5. 300 µl Serial Dilution Magazine (#650-08-96300) (Figure 1)
6. 300 µl D.A.R.T.’s (#5516)
7. 96-Well Polystyrene Microplate (#260836)
8. 0.5% Egg Shade Dye (Red, Yellow, Green, Blue McCormick [5ml/H2O])
9. Thermo Scientific™ Sorvall™ Legend™ microcentrifuge (#75002446)
10. Thermo Scientific™ Multiskan™ GO microplate spectrophotometer with Thermo Scientific™ SkanIt™ software (#51119200 or 51119300)
11. Thermo Scientific™ 8-Channel F1-ClipTip™ Pipette 300 µl (#4661140)
12. ClipTip Pipette Tips 300 µl (#94410513)
**Detailed Serial Dilution Programming Method**

The Versette is programmed using the ControlMate software supplied with the instrument.

**To prepare the plate:**

1. Using a Multidrop Combi, add 100 μl of diH2O into columns 2–12 of a 96-well flat bottom plate.
2. Using a 300 μl 8-Channel F1-ClipTip pipette, add a total of 300 μl of a combination of 0.5% egg shade dye solutions to columns A1–H1 in the same 96-well flat bottom plate; (detail of concentrations listed on page 2).
3. Place prepared 96-well flat bottom plate onto Stage 4 of the Versette.

**Creating the protocol sequence in the software:**

4. Select a Group step and name it “Serial Dilution Col 1 to Col 12”, with a loop of 11 iterations.
5. Add a Move step at Stage 4: well selection begins in Column A1 and ends in Column A11. Add an Aspirate step with 200 μl of sample using a predefined height “Aspirate”.
6. Add a Move step at Stage 4: well selection begins in Column A2 and ends in Column A12. Add a Dispense All step using a predefined height “Dispense”. (In our experiment, a dwell time of 1 second was also used.)
7. Add an Aspirate step for a 5 μl column of air by using a predefined height “Well Top” and a dwell time of 1 second.*
8. Select a Group step and name it “Mix”. Enter the value of 5 iterations for the loop field.*
9. Add an Aspirate step with 150 μl of sample using a predefined height “Aspirate”. Add a Dispense step using a predefined height “Dispense”; also use a “Specific volume” of 150 μl.
10. Add a Dispense step with a predefined height “Well Top” and a “Dispense All”.
11. Repeat across the plate for remaining columns A3–A12.
12. Select a Group step and name it “200 μl from Final Col 12 to Waste”. Move to Column A12 and aspirate 200 μl by using a predefined height “Aspirate”. Move to Stage 2 and eject the tips. Now each column incorporates only 100 μl of final volume.

*Refer to Figure 4

**Analyze the data:**

13. In our experiment, the completed 96-well plate was read at 405 nm using a Multiskan GO.
14. Results were calculated with SkanIt software, which is provided with Multiskan GO.

**Optimization Notes**

**Step 7:** Aspirating additional 5 μl of air ensures all of the sample is thoroughly cleared from the tip.

**Step 8:** (Optional Method) Instead of a mix, use a group command named “Mix” with individual Aspirate and Dispense steps to better define and optimize heights. A Mix command cannot predefine different heights with other specific features.

ControlMate software offers many additional options for optimizing your results, which may be required for certain types of liquid classes and conditions.

**Other optimization examples and why they are used:**

- **Air gap:** When used before an aspiration step, this gap created above the liquid during a dispense step assists in pushing the liquid column fully and completely out of the tip to ensure complete, accurate and consistent dispense. When used after an aspiration step, it ensures that the liquid in the tip does not leak during instrument movements or pauses due to volatility.

- **Blow out:** Moves the pipetting head pistons past the “zero volume” dispense point, pushing a small amount of air after the liquid. This command, in conjunction with the air gap, aids in pushing any remaining liquid from the tip. This is commonly used to overcome capillary action.

- **Dwell time:** Used to specify a period of time over which to leave the tips in the sample immediately after aspirate or dispense steps. This allows for equalizing air pressure and liquid movement inside the tips.

- **Speed:** This can be important when using low volumes or high viscosity liquids as the speed of the pipette head pistons, as well as other system motions, can be controlled to improve accuracy and precision.

- **Tip touch:** Removes excess liquid from tip orifice so it is not brought over to destination plate. This step causes the pipette tips to touch against a top side of a well after aspiration/dispensing to remove liquid which may have adhered to the side or bottom of the tips. Tip touch is essential for extremely low volume dispense accuracy.
Results

The graph (Figure 7) represents the serial dilution curve created with SkanIt software from the 405 nm absorbance readings. This curve shows how serial dilution was achieved by using several of the serial dilution optimization features available using the ControlMate software.

Figure 5: Completed serial dilution results.

Figure 7: 1:1.5 Serial dilution results average absorbance by column.

Conclusion

Serial dilutions included in many laboratory procedures can vary between diluting reagents, compounds or cells. Performing serial dilutions manually can be tedious and time-consuming, and can increase the risk of repetitive stress injuries (RSI’s) to the user. Errors may also occur with each sequential serial dilution step between columns, which can lead to less accurate and precise dispensing. Utilizing the Versette automated liquid handler for these serial dilutions helps to ensure efficiency and consistency, and eliminates the need for manual pipetting. It also saves time, as the protocols are completed much faster with minimal user intervention.

Figure 6: SkanIt software programming.

Figure 7: Completed serial dilution results.