Application Note

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Enabling Automated Serial Dilutions Using the Agilent Bravo BenchCel Workstation

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Introduction

Many research laboratory protocols require serial dilution of reagents or compounds for high-throughput screening experiments. For example, IC50 assays are commonly used to evaluate enzyme activity as part of the development procedures. These experiments require generating numerous standard curves that involve diluting proteins, small molecules, as well as other detection agents in large batches. The required experimental setup requires highly accurate and precise pipetting that can be streamlined using automated liquid handling equipment with serial dilution capabilities.

The Agilent Bravo Automated Liquid Handling Platform is ideally suited to handle an automated serial dilution workflow. It has nine standard deck positions that can hold any ANSI-standard microplate, and be customized with a variety of accessories to enable functionality such as heating, cooling, shaking, barcode reading, and tip washing. The Agilent Bravo Pipetting Head can accommodate volume transfers by column or row, from 300 nL to 200 µL. Further integration of the Agilent BenchCel Microplate Handler provides an efficient way to store and dispense source and destination microplates to increase throughput and facilitate more walk-away time. This Application Note describes how these two instruments, acting as an integrated Agilent Bravo BenchCel Workstation, can provide a complete solution for automating the creation of serial dilution microplates in column and row formats. Instrumentation is driven through a preconfigured Agilent VWorks Automation Control Software protocol that is easy to use and fully enabled for this application.

Experimental

Reagents

- Tartrazine (Acros Organics)
- Deionized (DI) water

Sample preparation

0.096 grams of Tartrazine was dissolved in 500 mL of DI water to create a 0.36 mM Tartrazine Solution standard.

Agilent VWorks Automation Control Software

To simplify startup for new users, preloaded protocols for automated serial dilution are provided with each Bravo BenchCel Workstation.

Equipment

The serial dilution workflow was carried out using an Agilent Bravo BenchCel Workstation System (Figures 1 and 2) consisting of:

- Agilent BenchCel 4R Microplate Handler with 660-mm Front Loading Racks
- Agilent Bravo Automation Liquid Handling Platform with Gripper
- Agilent 96LT Disposable Pipette Tip Head
- Agilent Bravo Deck Position Trash Chute
- Agilent 96LT 250 µL Disposable Pipette Tips
- Agilent 96 Well Manual Fill Reservoir
- 96-well polystyrene, clear flat bottom microplates (Greiner 655101)
- Nunc 96-deep well polypropylene, U bottom microplates (Thermo Fisher 260251)
- Thermo Scientific Varioskan Flash

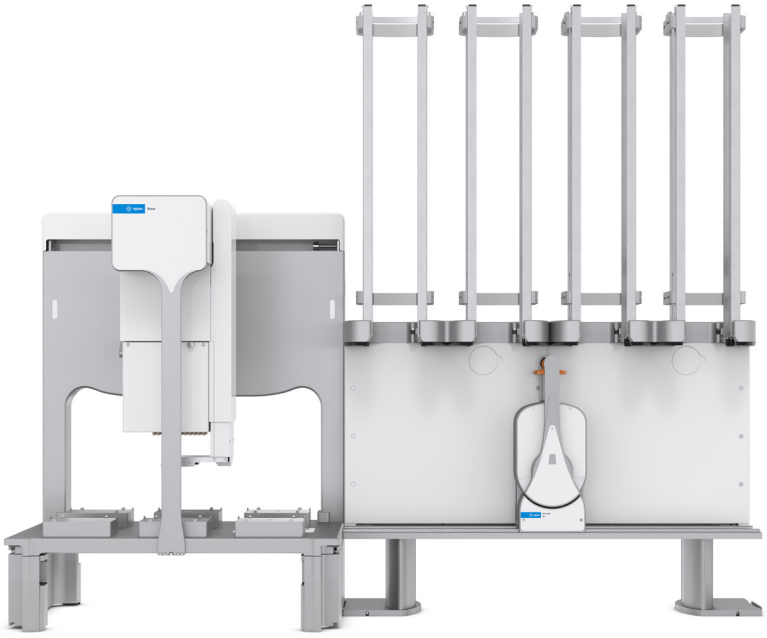

Figure 1. Agilent Bravo BenchCel Workstation System for Automated Serial Dilution.

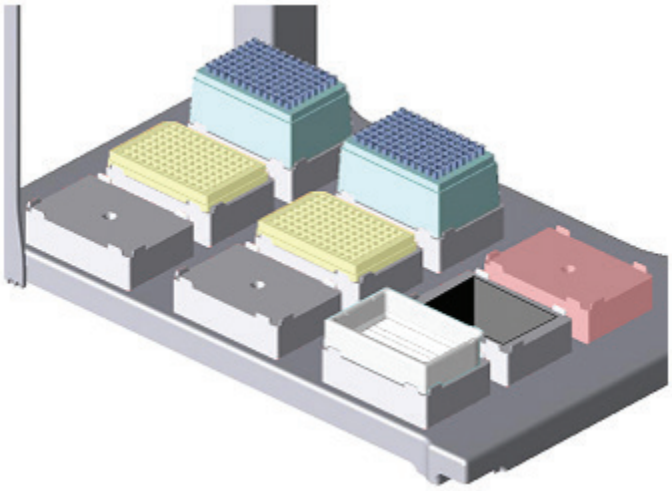

Figure 2. Agilent Bravo deck layout for automated serial dilution by column.

Serial Dilution Protocol (column-based)

Instrument setup

Prepare a 96 deep well microplate with 1 mL of tartrazine standard solution into each well (Sample Plate). Load into BenchCel Stacker 2.

1. Open VWorks Software and load the Serial Dilution Protocol.
2. Load 60 mL of DI water into 96 Well Manual Fill Reservoir (Bravo deck position 9).
3. Load an empty 96-well polystyrene microplate (Serial Dilution plate) on BenchCel Stacker 1.
4. Load 250 µL pipette tip boxes at Bravo deck positions 1 and 2.
5. Set up a liquid class for a 51–200 µL dispense in Agilent VWorks Automation Control Software.

Instrument workflow

1. Transfer Serial Dilution Plate from BenchCel Stacker 1 to Bravo deck position 5.
2. Transfer Sample Plate from BenchCel Stacker 2 to Bravo deck position 4.
3. Press 250 µL disposable pipette tips onto the 96LT Head at Bravo deck position 1.
4. Transfer DI water from 96 Well Manual Fill Reservoir into Serial Diluton Plate in Columns 2–12. Aspirate parameters are 6 mm from the bottom of the reservoir with a 2 µL pre-aspirate volume. Dispense parameters are 2 mm from the bottom of the plate, with a 2 µL blowout volume.
5. Unload the 250 µL pipette tips back to Bravo deck position 1.
6. Press eight 96LT 250 µL disposable pipette tips onto the last column of the head from Bravo deck position 2.
7. Mix Sample Plate before transferring into Serial Dilution Plate. Parameters are three mix cycles of 150 µL, 6 mm from the bottom of the plate with a 2 µL air gap.
8. Transfer 150 µL of Tartrazine solution from Sample Plate into Column 1 of the Serial Dilution Plate. Aspirate 2 mm from the bottom of the reservoir with a 2 µL pre-aspirate volume. Dispense 2 mm from the bottom of the plate, with a 2 µL blowout volume. Perform three mix cycles of 150 µL, 6 mm from the bottom of the plate with a 2 µL air gap.
9. Perform a 1:2 serial dilution (150 µL) from Columns 1–10. Aspirate a 2 µL pre-aspirate volume 2 mm from the bottom of the plate. Dispense 2 mm from the bottom of the plate, with a 2 µL blowout volume.
10. Discard 150 µL of excess volume from Column 10 along with the pipette tips on the Bravo Deck Position Trash (Bravo deck position 6).
11. Transfer Sample Plate back to BenchCel Stacker 4.
12. Transfer Serial Dilution Plate from Bravo deck position 5 to BenchCel Stacker 3.
13. Remove Serial Dilution Plate from BenchCel Stacker 3 and centrifuge at 1,800 rpm for 60 seconds to ensure consistent well menisci.
14. Perform an absorbance reading using the Thermo Scientific Varioskan Flash instrument.

Data processing

Absorbance measurements from each well were used to determine the precision of the transfer. Coefficient of variance (CV) calculations were made by dividing the standard deviation by the mean for each column/row. Accuracy ratio was calculated by averaging the absorbance reading of two adjacent column/rows where a pipette dilution occurred.

Results may vary depending on individual experimental methods and liquid class optimization.

Results and discussion

This proof-of-principle experiment was designed to demonstrate that the Agilent Bravo BenchCel Workstation can provide an automated solution for handling serial dilution workflows with batches of microplates. In this experiment, each Serial Dilution Plate has an estimated processing time of 10 minutes. This includes the time in which the Serial Dilution Plates are unloaded from the Agilent BenchCel Microplate Handler onto the Agilent Bravo Liquid Handling deck for the serial dilution steps, after which they are returned to the appropriate BenchCel stacker.

To demonstrate the precision and accuracy of the Bravo Liquid Handling Platform, the CV and accuracy ratio was calculated for three independent Serial Dilution Plates (Table 1). Overall, the average precision and accuracy ratio was demonstrated to be within 5.0 % of the expected measurement across the entire plate. A perfect serial dilution would have an accuracy ratio of 1:2.00 across the entire plate. The accuracy ratio of the plate improves with optimal pipetting parameters. For the column serial dilution experiment, Columns 9 and 10 were removed from data analysis as the absorbance readings on these two columns were very close to the background reading in Columns 11 and 12.

Table 1. Precision, accuracy ratio, and serial dilution time for three Serial Dilution Plates prepared by column.

Plate no.	CV/Column								Average precision	Time (sec/plate)	Accuracy ratio
	1	2	3	4	5	6	7	8			
1	1.3 %	1.0 %	2.3 %	1.4 %	2.9 %	2.8 %	3.4 %	6.9 %	2.7 %	568	1:1.95
2	1.4 %	1.7 %	1.8 %	1.6 %	1.5 %	2.5 %	4.5 %	4.4 %	2.4 %	579	1:1.95
3	0.6 %	1.4 %	1.8 %	3.3 %	2.2 %	3.1 %	5.2 %	8.6 %	3.3 %	700	1:1.95

Table 2. Precision, accuracy ratio, and serial dilution time for three Serial Dilution Plates prepared by row.

Plate no.	CV/Row						Average precision	Time (sec/plate)	Accuracy ratio
	1	2	3	4	5	6			
1	0.7 %	1.8 %	3.0 %	3.4 %	2.4 %	3.0 %	2.4%	517	1:1.89
2	0.5 %	1.6 %	2.6 %	2.5 %	2.8 %	4.7 %	2.5%	455	1:1.89
3	0.7 %	1.8 %	2.5 %	2.2 %	3.5 %	3.3 %	2.3%	453	1:1.89

Since the Bravo Liquid Handling Platform can also perform serial dilutions by rows, a separate experiment was performed to confirm the consistency in this orientation. Table 2 shows that by applying the same pipetting parameters, high precision and accuracy was possible with preparing serial dilutions by rows.

The use of optimal pipetting parameters for mixing steps before or after a dilution step contributes to a low CV and a tight accuracy ratio. The following parameters are key to obtaining precise and accurate mixing when using a liquid handling platform for creating serial dilutions:

- Maximize mix volumes
- Maximize mixing speed
- Maximize the area traversed by the tip retraction/extension

Conclusion

The Agilent Bravo BenchCel Workstation demonstrates a quick and reliable solution for processing serial dilutions into multiple microplates across columns and rows. These experiments show that optimized pipetting parameters can display accuracy and precision when preparing serial dilution samples. Additionally, the workflow was performed with a short process time without any pipette tip changes, minimal number of mix cycles, and no required intermediary wash steps. The automation protocol created in the Agilent VWorks Automation Controls Software for this experiment provides a simple template for new users that can easily be customized. Overall, the Bravo BenchCel Workstation provides a complete solution for automating serial dilutions for microplates in high-throughput research environments.

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DE.5949074074

Printed in the USA, October 29, 2020
5991-8090EN

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