AssayMAP Protein Sample Prep Workbench

Serial Dilution v3.0 Quick Start Guide

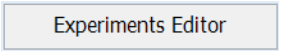
This guide is for users who have been trained in the proper use of the AssayMAP Bravo Platform and understand the safety guidelines in the *Bravo Platform Safety and Installation Guide*. The procedures in this guide require the Protein Sample Prep Workbench. You can find more detailed instructions by going to the Literature Library in the Protein Sample Prep Workbench.

Before you start

Each workbench application and utility has an Experiment Settings section that allows you to select an experiment ID and a method.

- An *experiment ID* is a database record that captures the steps executed and the settings used during each run of an application or utility. Any errors that may have occurred during a run are also recorded.

  To create an experiment ID, you open the Experiments Editor by clicking

  in any Workbench app or utility. For details, go to the Literature Library and open *Using the Protein Sample Prep Workbench*. In the browser that opens, click **Using Experiment IDs**.
- A *method* is a comprehensive collection of saved settings for an application or utility, which you can use to run the application or utility.

Experiment IDs and methods are required for compliance-enabled VWorks editions and optional for noncompliance-enabled VWorks editions.

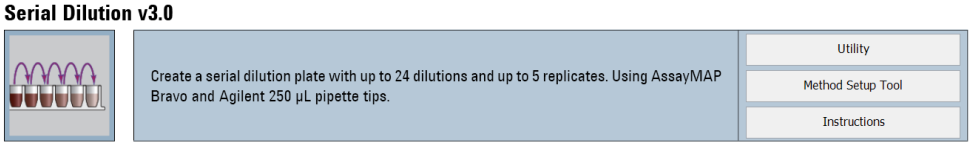
VWorks edition	Experiment ID and method selection
VWorks Plus	Required
VWorks Standard	Optional

Step 1. Design the Serial Dilution method

VWorks Plus only. Administrator or technician privileges are required to create or modify methods. In addition, you must save the method before you can run it.

***To open the Serial Dilution Method Setup Tool*:**

In the **Utility Library**, locate the **Serial Dilution v3.0** banner, and then click **Method Setup Tool**.

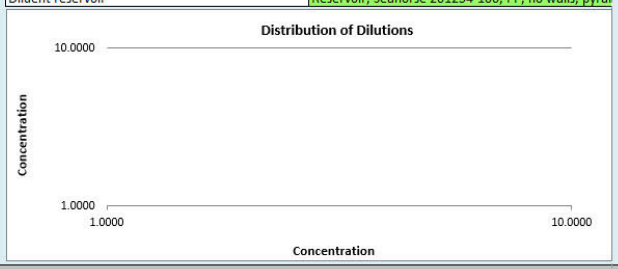


The Method Setup Tool has 6 distinct steps. The following figure and table provide an overview. For in-depth guidelines, see the *Serial Dilution User Guide*.

Serial Dilution: Method Setup Tool v3.0 — Agilent Technologies

Step 1) Set dilution parameters

Input		Value
Final concentration of dilution 1	(mg/mL)	0
Optional volume (µL) to be added		0
Final volume (µL) of dilution series		0
Direction of dilution		top to bottom
Number of replicates		1
Mix cycles after each dilution		10
Change pipette tips after each dilution		Yes
Pre-wet pipette tips before transfer		Yes
Blowout volume (µL)		5
Liquid class		<Automatic>
Serial dilution plate		96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
Diluent reservoir		Reservoir, Seahorse 201254-100, PP, no walls, pyra



Step 2) Define dilution concentrations — Clear All

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

Step 3) Select the starting well of the dilution series — Clear Steps 3 and 4 Only

Serial Dilution Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Select Starting Well

Step 4) Calculate the sample and diluent volumes

Sample Volume

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Calculate Volumes

Diluent Volume

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Step 5) Correct these errors:

Step 6) Create the method file

Create Serial Dilution Method

Step 7) Prepare serial dilution plates

Table Overview of steps in Method Setup Tool

Step		Description	
1	Set dilution parameters	Enter values for the following inputs:	Default value (Range)
		Final concentration of dilution 1	0 (>0)
		Optional volume (µL) to be added	0 (0 to < Final volume of dilution series)
		Final volume (µL) of dilution series	0 (5–500)
		Direction of dilution	top to bottom (top to bottom, bottom to top, left to right, right to left)
		Number of replicates	1 (1–5)
		Mix cycles after each dilution *Note:* See the user guide for advice about the number of mix cycles when the volume is greater than approximately 300 µL.	10 (0–100)
		Change pipette tips after each dilution	Yes (No/Yes)
		Pre-wet pipette tips before transfer	Yes (No/Yes)
		Blowout volume (µL)	5 (0–50)
		Liquid class	Automatic (preset or custom)
		Serial dilution plate	See user guide
		Diluent reservoir	See user guide
2	Define dilution concentrations	Enter the final target concentration for each serial dilution step.	
3	Select the starting well of the dilution series	Click **Select Starting Well**, and then click the cell in the **Serial Dilution Plate** map where the replicate 1 of dilution 1 will be located. The setup tool will display the plate layout based on the input provided in steps 1 and 2.	
4	Calculate the sample and diluent volumes	Click **Calculate Volumes**. The setup tool calculates the required volumes of sample and diluent based on the input provided in steps 1 to 3.	
5	Correct these errors	Resolve any errors that the setup tool highlights.	
6	Create the method file	Click **Create Serial Dilution Method**. After you save the method, the Method Setup Tool closes.	

IMPORTANT If you change anything in step 1 or 2 after completing step 3 or 4, make sure that you click **Clear Steps 3 and 4 Only** and redo steps 3 and 4.

Step 2. Prepare the sample and diluent

Prepare the serial dilution plate with the initial dilution and diluent reservoir plate to match the volumes and well positions specified in the method created using the Serial Dilution Method Setup Tool. You can view the method preparation instructions in the Serial Dilution utility when you select the method. Alternatively, you can export the method and view it in Microsoft Excel. See the *Serial Dilution v3.0 User Guide* for details.

If necessary, use the Reformatting utility to transfer the samples into the appropriate labware type.

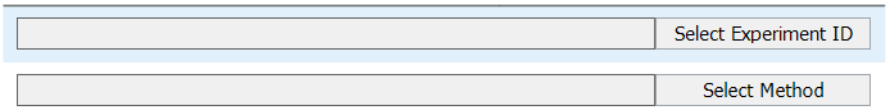
To minimize evaporation, fill the labware immediately before run time or keep them covered until you run the protocol.

Step 3. Prepare the system

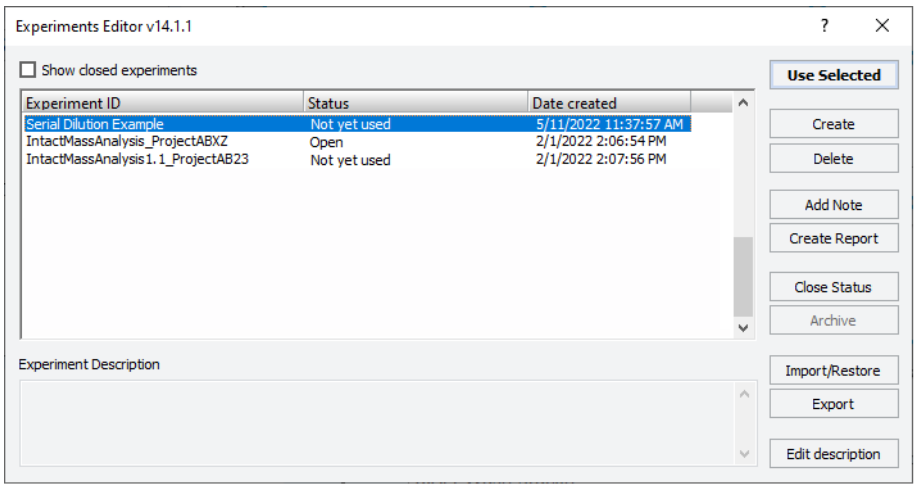
To prepare the system:

1. Check the levels of the wash station source and waste carboys, and fill or empty as required.
2. If you have not already done so, turn on the AssayMAP Bravo Platform and accessories, and start the Protein Sample Prep Workbench.
3. Open the **System Startup/Shutdown** utility.

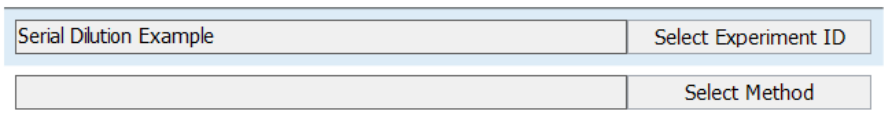
   Note: For detailed instructions, see the user guide for this utility.
4. If applicable, click **Select Experiment ID** to open the Experiments Editor.



5. In the **Experiments Editor**, select the **Experiment ID** that you want to use to capture the steps performed during this utility run, and then click **Use Selected**.



The Experiments Editor closes.



6 In the form, click **Select Method** to locate and select a method for this utility.

In the **Open File** dialog box, select the method, and click **Open**.

7 Confirm that the labware and accessories on the AssayMAP Bravo deck match the display in the **Deck Layout** area of the form.

8 Click 

to start the run.

WARNING **The Bravo head and tie bar will move during the Bravo Startup protocol. To prevent injury, keep clear of the device while it is in motion.**

9 During the Startup protocol, verify that all the wash station chimneys have liquid flowing through them. If liquid is not flowing through the chimneys, see the *96 Channel Wash Station Maintenance Guide* for troubleshooting guidelines.

Step 4. Run the utility

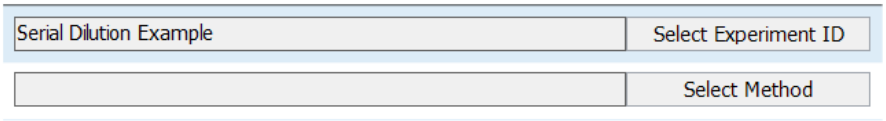
To run the Serial Dilution utility:

1 Open the **Serial Dilution** utility.

Note: For detailed instructions, see the user guide for this utility.

2 If applicable, click **Select Experiment ID** to open the Experiments Editor.

3 In the **Experiments Editor**, select the **Experiment ID** that you want to use to capture the steps performed during this utility run, and then click **Use Selected**.

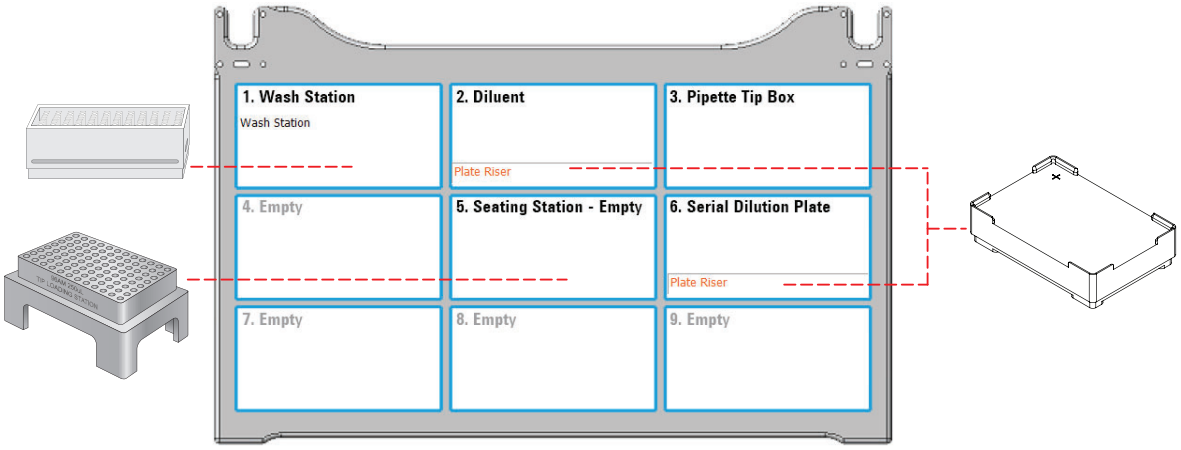


4 In the form, click **Select Method** to select and load the method for this utility.

WARNING **The probes of the Bravo 96AM Head are sharp and can scratch you if they brush across your hand. A probe scratch can expose you to any contaminants remaining on the probes. Be careful to avoid touching the probes.**

5 Ensure that the following items are securely in place at their respective AssayMAP Bravo deck locations:

- Bravo Plate Riser at deck locations 2 and 6.
- The empty 96AM Cartridge & Tip Seating Station at deck location 5.



CAUTION **To prevent a potential collision, ensure that no thermal plate insert is on the Peltier Thermal Station installed at deck location 4.**

6 Place a tip box full of fresh 250-µL pipette tips at deck location 3, place the serial dilution plate on the plate riser at deck location 6, and place the diluent reservoir plate on the plate riser at deck location 2.

Ensure that the labware on the deck exactly matches the **Deck Layout** in the form.

CAUTION **Incorrect labware selections and improperly seated labware can cause hardware collisions, resulting in equipment damage. Ensure that the selections in the method exactly match the physical labware present on the Bravo deck. Also ensure that all labware are properly seated within the alignment features of their respective platepads.**

7 Click Run Protocol to start the run.

To monitor the progress of the run, check the **Status** box in the upper right corner of the form.

Step 5. Clean up after each run

To clean up after the run:

1 Remove used labware from the deck.

2 Discard leftover reagents appropriately.

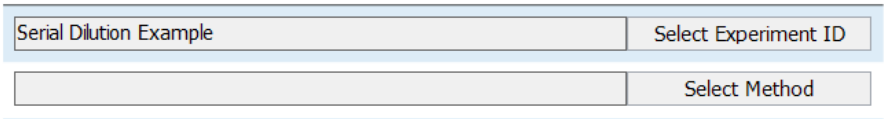
3 *Optional*. Conduct stringent washing of the syringes:

a Open the **Syringe Wash** utility  .

Note: For detailed instructions, see the user guide for this utility.

b If applicable, click **Select Experiment ID** to open the Experiments Editor.

c In the **Experiments Editor**, select the **Experiment ID** that you want to use to capture the steps performed during this utility run, and then click **Use Selected**.



d Click **Select Method** to select and load the method for this utility.

e Confirm that the labware and accessories on the AssayMAP Bravo deck match the display in the **Deck Layout** area of the form.

f Click Run Protocol to start the run.

Step 6. Add optional volume

If the Serial Dilution method specifies an Optional volume to be added, run the Reformatting utility to add the specified volume to the serial dilution plate.

Step 7. Shut down at end of day

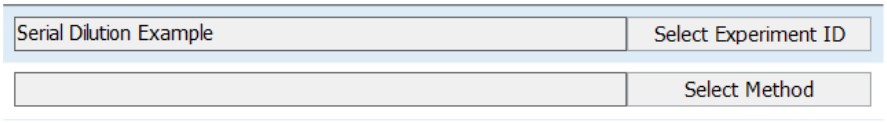
To shut down at the end of the day:

1 Open the **System Startup/Shutdown** utility**.**

Note: For detailed instructions, see the user guide for this utility.

2 If applicable, click **Select Experiment ID** to open the Experiments Editor.

3 In the **Experiments Editor**, select the **Experiment ID** that you want to use to capture the steps performed during this utility run, and then click **Use Selected**.



4 Click **Select Method** to select and load the method for this utility.

5 Remove everything from the deck except the 96AM Wash Station (deck location 1), the 96AM Cartridge & Tip Seating Station (deck location 2), and if applicable, the Syringe Storage Liquid (deck location 7).

6 Click Run Shutdown.

7 After the Shutdown protocol has completed, turn off the power at the AssayMAP Bravo Platform and the accessories.

8 Close the Protein Sample Prep Workbench software.

Utility overview

The following table summarizes the basic movements of the AssayMAP Bravo Platform during the Serial Dilution protocol.

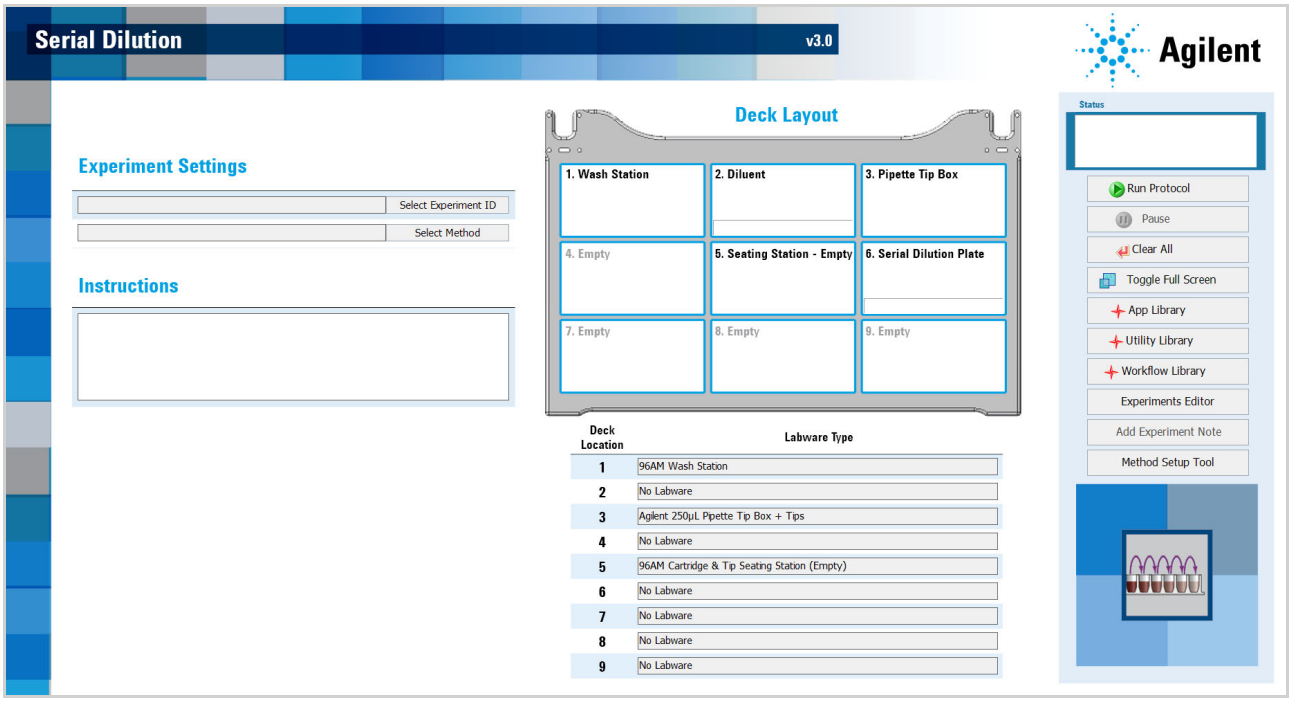


Table **Automation movements during the protocol**

Protocol process*	Process name	Process description
1	Syringe Wash	Washes the external part of the syringe at the wash station (deck location 1).

Protocol process*	Process name	Process description
2	Syringe Drying	Performs 4 syringe aspirate-and-dispense cycles above the wash station (deck location 1) to cycle air in and out of the syringes. The syringes move over the chimneys after each cycle to remove any droplets that were pushed out of the syringes during the cycle.
3	Initial Tip Transfer	Transfers all 96 250-µL pipette tips from the tip box (deck location 3) to the 96AM Cartridge & Tip Seating Station (deck location 5).
4	Offset Head Tip Pickup	Picks up between 1 to 5 pipette tips, depending on the number of replicates, from the next available column or row of pipette tips in the 96AM Cartridge & Tip Seating Station (deck location 5). The column or row orientation depends on the dilution direction specified in the method, for example, a top-to-bottom or left-to-right dilution series.
5	Diluent Transfer	Moves to the diluent reservoir (deck location 2). Aspirates an air gap if a blowout is specified. Prewets the pipette tips, if specified. Aspirates diluent into the pipette tips, and then dispenses the diluent into the designated wells in the serial dilution plate (deck location 6). Performs a blowout, if specified. Repeats the aspirate-and-dispense process using the same pipette tips until all the wells in the serial dilution plate have the specified amount of diluent. If a blowout is specified, aspirates an air gap at the start of each aspirate task and performs a blowout and tip touch after each dispense task.
6	Change Tips	Ejects the used pipette tips into the tip box (deck location 3). Picks up between 1 to 5 pipette tips, depending on the number of replicates, from the next available column or row of pipette tips in the 96AM Cartridge & Tip Seating Station (deck location 5).
7	Serial Dilution Transfer	Moves to the lowest concentration serial dilution step available in the serial dilution plate (deck location 6). Aspirates an air gap if a blowout is specified. Prewets the pipette tips, if specified. Aspirates the sample into the pipette tips, and then dispenses the sample into the next set of dilution wells in the serial dilution plate. Mixes the well contents for the set number of cycles, if specified. Performs a blowout, if specified.
8	Optional Change Tips	Ejects the used pipette tips into the tip box (deck location 3), and then picks up between 1 to 5 pipette tips, depending on the number of replicates, from the next available column or row of pipette tips in the 96AM Cartridge & Tip Seating Station (deck location 5). If the change tips option is not specified, the same set of pipette tips are used for all the serial dilution transfers.

Protocol process*	Process name	Process description
9	Additional Serial Dilution Transfers	Moves to the lowest concentration serial dilution step available in the serial dilution plate (deck location 6). Aspirates an air gap if a blowout is specified. Prewets the pipette tips, if specified. Aspirates the sample into the pipette tips, and then dispenses the sample into the next set of dilution wells in the serial dilution plate. Mixes the well contents for the set number of cycles, if specified. Performs a blowout, if specified, followed by a tip touch on the east and west sides of the wells. Repeats processes 7 and 8 until the serial dilution series is complete.
10	Final Tip Ejection	Ejects the used pipette tips into the tip box (deck location 3).
11	Park Head	Moves to the parked position above the wash station at deck location 1.

*For more in-depth descriptions of the robotic movements executed during a run, see the *Assay development guidelines* topic in the user guide.

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