AssayMAP Protein Sample Prep Workbench

On-Cartridge Reaction v2.0 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 Experiments Editor 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 your run

This application uses cartridges that have been prepared during a preceding application run (for example, Immobilization or Affinity Purification).

Use the **On-Cartridge Reaction Reagent Volume Calculator** to:

- Determine reagent volume preparation requirements.
- Ensure the labware selections are consistent with volume requirements.

For in-depth assay development guidelines, see the *On-Cartridge Reaction v2.0 Users Guide* in the Literature Library of the Protein Sample Prep Workbench.

Step 2. Prepare reagent plates

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

CAUTION **A small reagent volume excess is required in all labware types to ensure proper volume transfer.**

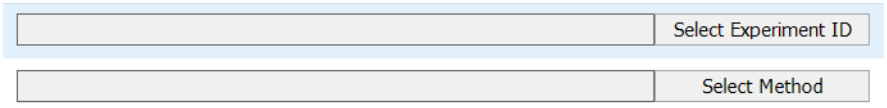
Use the Reagent Volume Calculator to automatically include excess volume, or look up the recommended value for each allowable labware type in the *AssayMAP Labware Reference Guide*, which is available in the Literature Library page of the workbench.

Step 3. Prepare the system

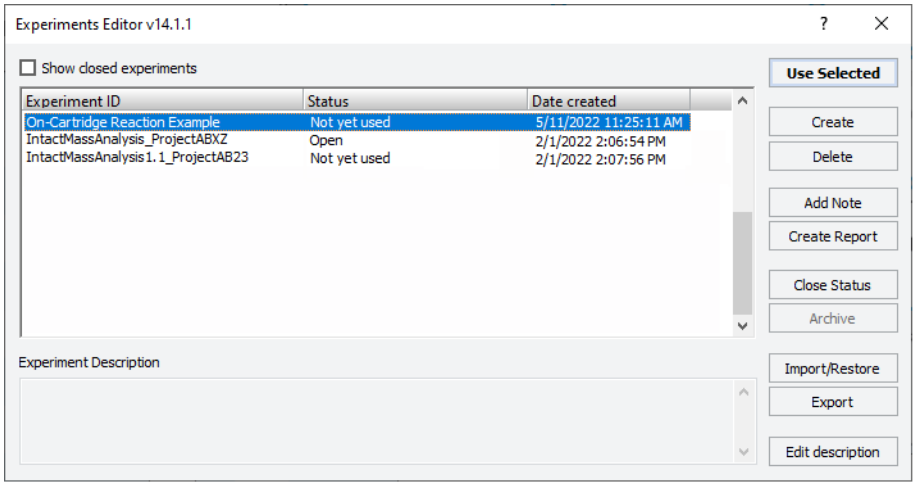
To prepare the system:

1. Check the levels of the wash station source and waste carboys. Then, fill or empty them 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.

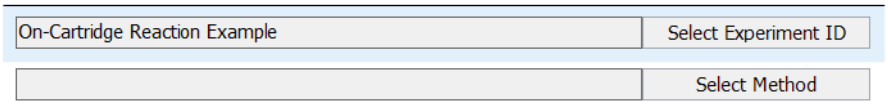
   Note: For detailed instructions, see the user guide for this utility.
4. If applicable, click **Select Experiment ID** to open the Experiments Editor.



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.

CAUTION **To avoid a hardware crash and equipment damage, ensure that the wash station contains the white wide-bore chimneys when using the AssayMAP 25 µL cartridges.**

Note: The wash station wide-bore chimneys work for both 5-µL and 25-µL cartridges and are standard on wash stations purchased in 2020 onward. The wide-bore chimneys are white plastic, whereas the normal-bore chimneys are a semi-clear plastic. For details, see the *96 Channel Wash Station Maintenance Guide*.

8 Click Run Startup 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. Set up the cartridges

If necessary, use the Cartridge Transfer utility to set up cartridges in the 96AM Cartridge & Tip Seating Station.

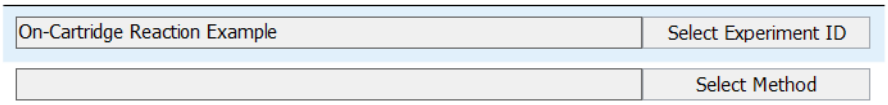
To set up the cartridges:

1 Open the **Cartridge Transfer** 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.

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

6 Click Run Protocol to start the run.

IMPORTANT The On-Cartridge Reaction application requires cartridges that have been prepared using another application where sample was bound to the cartridge resin. You should use the cartridges immediately after the sample is loaded on the resin, or store them in a 96AM Cartridge Rack with liquid in the Receiver Plate wells to avoid drying out. If the resin was allowed to dry out, the cartridges should not be used.

Step 5. Run the application

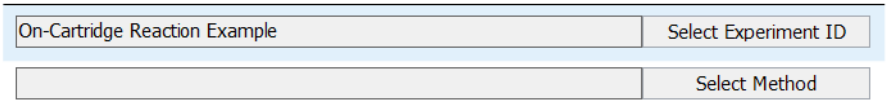
To run the application:

1. Open the **On-Cartridge Reaction v2.0** app.

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

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 application run, and then click **Use Selected**.



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

   Note: Agilent provides a method with default settings for each cartridge size. Each default method file name has the cartridge size as a prefix.

   To modify the selected method, proceed to step 5. Otherwise, go to step 6.

5. To create or modify a method:

   VWorks Plus. Administrator or technician privileges are required to create or modify methods.

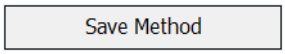
   a. In the **Application Settings** area, specify the cartridge settings:

   - Select the cartridge size from the **Number of Full Columns of** list: **5 µL Cartridges** or **25 µL Cartridges**.
   - In the box, type the number of full columns in the cartridge holder at deck location 2. Default: 1 (Range: 1–12).

   The position of the columns of cartridges in the tip seating station must match the positions of the samples and solutions in the plates on the deck. For details, see the application user guide.

   b. Select the remaining **Application Settings**. For help, see the following Application Settings section.

   c. In the **Labware Table** of the form, select the labware for your run.

   d. To save the method, click 

. In the **Save File As** dialog box, type the file name and click **Save**.

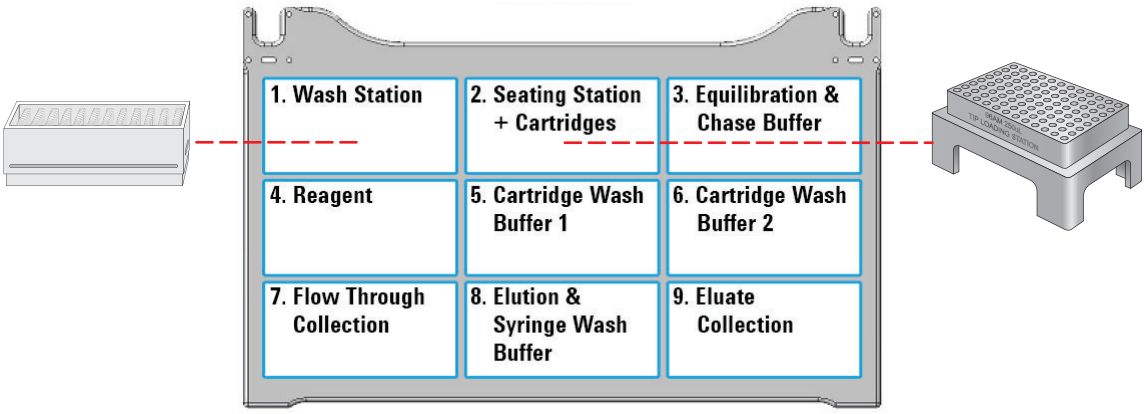
   Note: Agilent recommends that you use the cartridge size (5 µL or 25 µL) as a prefix to the name so that you know if the method matches the cartridge size in use.

   VWorks Plus. You must save the method before you can run it.

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.**

Note: The Greiner and BioRad PCR plates are not compatible with the 25 µL cartridges at deck locations 4, 7, and 9.

6 Ensure the accessories, filled reagent plates, and collection plates are at the assigned deck locations, as shown in the **Deck Layout** of the form.



CAUTION **Incorrect labware selections and improperly seated labware can cause hardware collisions, resulting in equipment damage. Ensure that the selections in the Labware Table 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 6. Clean up after each run

To clean up after the run:

1 Remove used labware from the deck.

2 Discard leftover reagents appropriately.

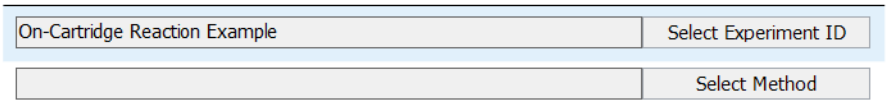
3 *Optional*. Conduct stringent washing of the syringes:

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.

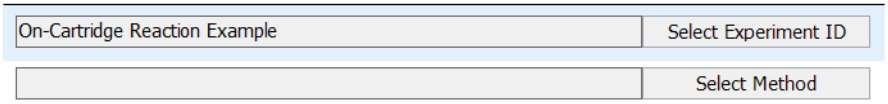
Step 7. Shut down at end of day

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 

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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.

Application Settings

The following table provides an overview of the Application Settings section in the On-Cartridge Reaction v2.0 app.

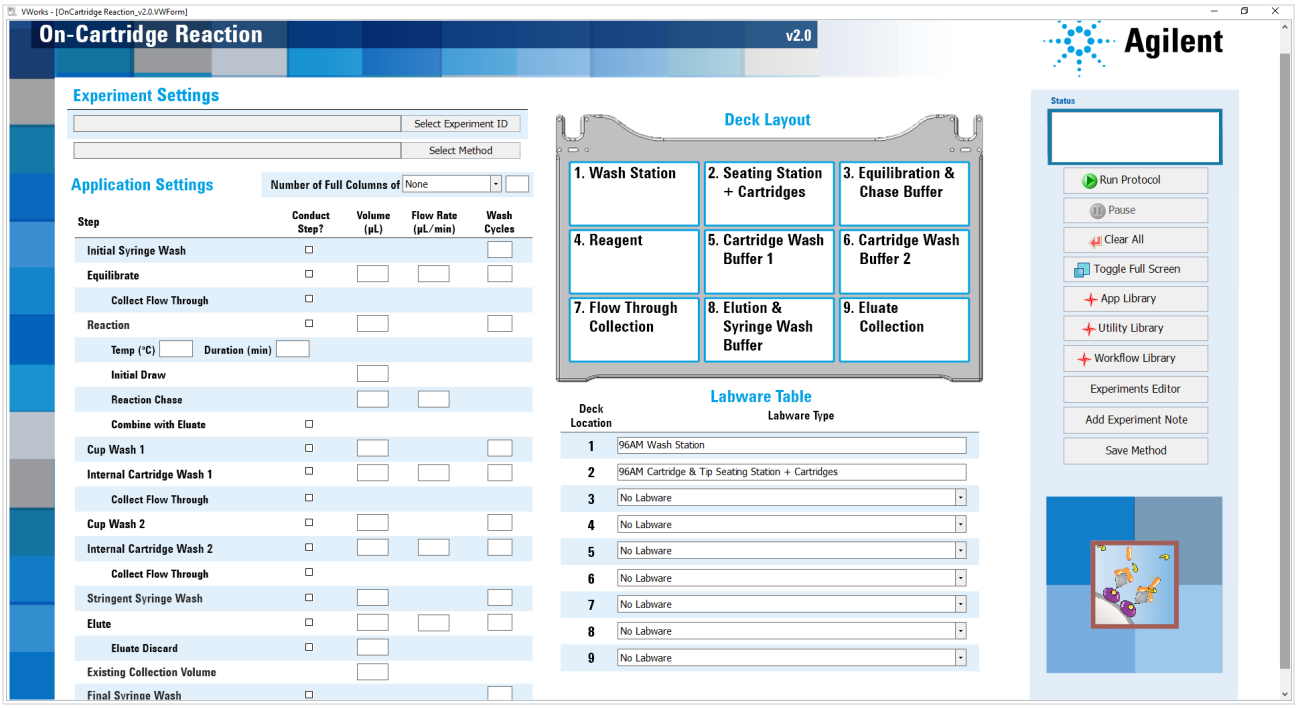

Table **Application Settings overview**

Steps*	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Initial Syringe Wash	Washes syringes at the wash station (deck location 1).	5 µL:	–	–	3
		25 µL:	–	–	3
		Range:	–	–	0–10
Equilibrate	Aspirates the Equilibration Buffer (deck location 3) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1) or into Flow Through Collection (deck location 7).	5 µL:	50	10	1
		25 µL:	250	10	1
		Range:	0–250	0.5–500	0–10
Collect Flow Through	If selected, collects the Equilibrate flow-through at Flow Through Collection (deck location 7). If not selected, discards the equilibration flow-through at the wash station (deck location 1).	–	–	–	–

Steps*	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Reaction	Aspirates the Reagent (deck location 4) through the cartridges in two steps (see note), followed by aspirating a chase volume. The Reaction flow-through and chase volume are collected at Flow Through Collection (deck location 7), unless Combine with Eluate is selected. The sum of the Reaction volume and the Reaction Chase volume must be less than 250 µL.	5 µL:	6	See note.	3
		25 µL:	30		3
		Range:	0–250		0–10
	Note: The initial draw volume is aspirated at 10 µL/min. Any additional volume (reaction volume minus the initial draw volume) is aspirated at a flow rate appropriate to satisfy the Duration setting.				
Tempera-ture	Specifies the set point temperature of the Peltier Thermal Station at deck location 4 during the Reaction step. The temperature in the cartridge will be less than this setting. See the user guide for more details.	5 µL:	Temperature: 25 °C		
		25 µL:	25 °C		
		Range:	4–110 °C		
Duration	Specifies the total length of time to aspirate the Reagent (deck location 4) through the cartridges.	Range:	Time (m): 30 (0–180)		
Initial Draw	Specifies the initial draw volume, which is aspirated at 10 µL/min. Any additional volume (reaction volume minus the initial draw volume) is aspirated at a flow rate appropriate to satisfy the Duration setting.	5 µL:	4	10	–
		25 µL:	20	10	–
		Range:	0–250	–	–
Reaction Chase	Aspirates the Chase Buffer (deck location 3) through the cartridges, to flush soluble reaction products into the syringes. This step occurs immediately after the aspiration of Reagent, combining the soluble reagent products and chase buffer within the syringes.	5 µL:	15	5	–
		25 µL:	75	5	–
		Range:	0–250	0.1–500	–
Combine with Eluate	If selected, collects the soluble reaction products at Eluate Collection (deck location 9). If not selected, collects the soluble reaction products at Flow Through Collection (deck location 7).	–	–	–	–
Cup Wash 1	Rinses the cartridge cups with the Cartridge Wash Buffer 1 (deck location 5), and then discards the liquid into the wash station (deck location 1).	5 µL:	25	–	3
		25 µL:	25	–	3
		Range:	0–100	–	0–10

Steps*	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Internal Cartridge Wash 1	Aspirates the Cartridge Wash Buffer 1 (deck location 5) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1) or Flow Through Collection (deck location 7).	5 µL:	50	10	3
		25 µL:	250	10	3
		Range:	0–250	0.5–500	0–10
Collect Flow Through	If selected, collects the Internal Cartridge Wash 1 flow-through at Flow Through Collection (deck location 7). If not selected, discards the Internal Cartridge Wash flow-through at the wash station (deck location 1).	–	–	–	–
Cup Wash 2	Rinses the cartridge cups with the Wash Buffer 2 (deck location 6) and discards the liquid into the wash station (deck location 1).	5 µL:	25	–	3
		25 µL:	25	–	3
		Range:	0–100	–	0–10
Internal Cartridge Wash 2	Aspirates the Cartridge Wash Buffer 2 (deck location 6) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1) or Flow Through Collection (deck location 7).	5 µL:	50	10	3
		25 µL:	250	10	3
		Range:	0–250	0.5–500	0–10
Collect Flow Through	If selected, collects the Internal Cartridge Wash 2 flow-through at Flow Through Collection (deck location 7). If not selected, discards the Internal Cartridge Wash 2 flow-through at the wash station (deck location 1).	–	–	–	–
Stringent Syringe Wash	Aspirates the Syringe Wash Buffer (deck location 8), and then discards the liquid into the wash station (deck location 1).	5 µL:	50	–	2
		25 µL:	50	–	2
		Range:	0–250	–	0–10
Elute	Aspirates the Elution Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into Eluate Collection (deck location 9).	5 µL:	25	5	1
		25 µL:	125	5	1
		Range:	0–250	0.1–500	0–10
Eluate Discard	If selected, a specified initial volume of Eluate will be dispensed through the cartridges, and then discarded at the wash station (deck location 1).	5 µL:	0	–	–
		25 µL:	0	–	–
		Range:	0–250	–	–
Existing Collection Volume	Specifies the volume of liquid present in the Eluate Collection plate (deck location 9) at the beginning of the run.	5 µL:	0	–	–
		25 µL:	0	–	–
		Range:	0–1000	–	–
Final Syringe Wash	Conducts the specified number of internal syringe washes at the wash station (deck location 1).	5 µL:	–	–	3
		25 µL:	–	–	3
		Range:	–	–	0–10

Steps*	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles

*Practical value ranges for the steps listed in this table and factors to consider when changing the default values can be found in the user guide for this application. Go to the *Assay development guidelines and protocol notes* topic and see the *Protocol stepwise guidelines* section.

A complete list of the robotic movements executed during a run can also be found in the user guide in the *Assay development guidelines and protocol notes* topic. See the *Automation movements during the protocol* section for details.

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