AssayMAP Protein Sample Prep Workbench

On-Cartridge Reaction v1.1 Quick Start Guide

This guide is intended 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 quick start guide require the Protein Sample Prep Workbench and VWorks Automation Control software. See the user guide to verify the required software versions.

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 v1.1 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 v1.1 Users Guide* in the Literature Library of the Protein Sample Prep Workbench.

Note: This application has not yet been optimized for the 25 µL cartridges.

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, 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 **Utility Library**, and then open the **System Startup/Shutdown** utility  .
4. Click **Run Startup** to prepare the system for 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.

5 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.

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*.

Step 4. Run the application

To run the application:

1 If necessary, run the **Cartridge Transfer** utility to set up the cartridges  in the Cartridge & Tip Seating Station.

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.

2 Open the **On-Cartridge Reaction v1.1** app.

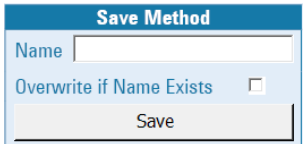
3 Under **Select Method**, click ..., locate and select the method file. Click **Load** to display all the settings associated with the selected method.

The On-Cartridge Reaction method storage location is C:/VWorks Workspace/Methods/AM OnCartridge Reaction v1.1.

To modify the selected method, proceed to step 4. Otherwise, go to step 5.

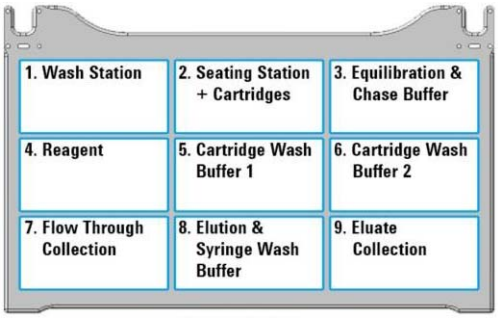
4 To create or modify a method:

- **a** Select the **Application Settings**. For help, see "Application Settings" on page 3.
- **b** In the **Labware Table** of the app interface, select the labware for your run.
- **c** In the **Save Method** area, specify the method **Name**, and if applicable, select **Overwrite if Name Exists**. Click **Save**.



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 when placing the labware on the Bravo deck.**

5 Place the filled reagent plates and collection plates at the assigned deck locations, as shown in the **Deck Layout** of the app interface.

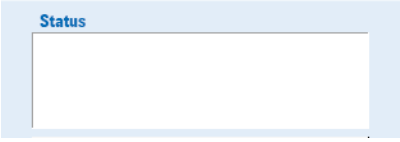


CAUTION **Improperly seated labware can cause a hardware collision, resulting in equipment damage. Ensure that all labware are properly seated within the alignment features of their respective platepads.**

CAUTION **Incorrect labware selections can cause a hardware collision, resulting in equipment damage. Ensure that the selections in the Labware Table exactly match the physical labware present on the Bravo deck.**

6 Click **Run Protocol** to start the run.

You can monitor the progress of the run and the run time in the **Status** box located 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*. To conduct stringent washing of the syringes, run the **Syringe Wash** utility  .

Step 6. Perform a shutdown if system will be idle for 2 hours or longer

To perform a shutdown:

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

2 Remove everything from the deck except the 96AM Wash Station (deck location 1), empty Cartridge & Tip Seating Station (deck location 2), and optionally, the wash solution (deck location 7). Click **Run Shutdown**.

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

4 Close the Protein Sample Prep Workbench software.

Application Settings

The following figure and tables provide an overview of the Application Settings section in the On-Cartridge Reaction v1.1 app.

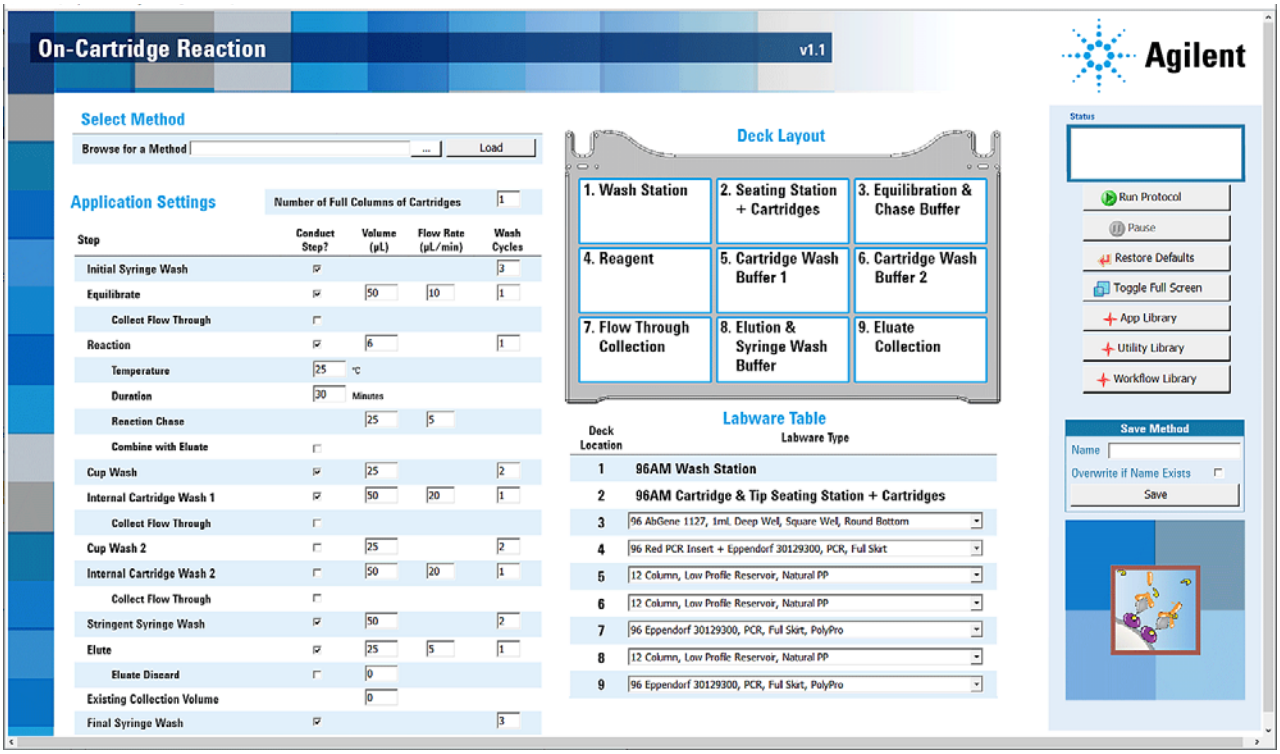


Table **Application Settings overview**

Setting	Description	Default value (range)
Number of Full Columns of Cartridges	Specifies the number of full columns in the Cartridge & Tip Seating Station at deck location 2.	1 (1–12)

Steps	Description	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Initial Syringe Wash	Washes all 96 syringes at the wash station (deck location 1).	–	–	3 (1–10)
Equilibrate	Aspirates the Equilibration Buffer (deck location 3) into the syringes, mounts the cartridges, and then dispenses a specified volume through the cartridges into the wash station (deck location 1) or into Flow Through Collection (deck location 7).	50 (0–250)	10 (0.5–500)	1 (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	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. *Note:* Initially, 4 µL is aspirated at 10 µL/min. Any additional volume is aspirated at a flow rate appropriate to satisfy the Duration setting.	6 (4–100)	See note.	1 (0–10)
Temperature	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.	Temperature (°C): 25 (4–100)		
Duration	Specifies the total length of time to aspirate the Reagent (deck location 4) through the cartridges.	Time (m): 30 (1–180)		
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.	25 (0–100)	5 (0.5–500)	–
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 Wash Buffer 1 (deck location 5) and discards the liquid into the wash station (deck location 1).	25 (5–100)	–	2 (1–10)
Internal Cartridge Wash 1	Aspirates the Wash Buffer 1 (deck location 5) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1), unless Collect Flow Through is selected, which dispenses the flow-through to Flow Through Collection (deck location 7).	50 (0–250)	20 (0.5–500)	1 (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).	25 (5–100)	–	2 (1–10)
Internal Cartridge Wash 2	Aspirates the Wash Buffer 2 (deck location 6) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1), unless Collect Flow Through is selected, which dispenses the flow-through into Flow Through Collection (deck location 7).	50 (0–250)	20 (0.5–500)	1 (0–10)

Steps	Description	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
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 buffer from Syringe Wash Buffer (deck location 8) into the syringes, and then discards the liquid into the wash station (deck location 1).	50 (0–250)	–	2 (1–10)
Elute	Aspirates the buffer from Elution Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into Eluate Collection (deck location 9).	25 (0–250)	5 (0.1–500)	1 (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).	0 (0–25)	–	–
Existing Collection Volume	Specifies the volume of liquid present in the Eluate Collection plate (deck location 9) at the beginning of the run.	0 (0–300)	–	–
Final Syringe Wash	Conducts the specified number of internal syringe washes at the wash station (deck location 1).	–	–	3 (1–10)

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