AssayMAP Protein Sample Prep Workbench

Affinity Purification: Aspiration Mode 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 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

Use the **Affinity Purification: Aspiration Mode Reagent Volume Calculator** to:

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

For in-depth assay development guidelines, see the *Affinity Purification: Aspiration Mode v3.0 User 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.

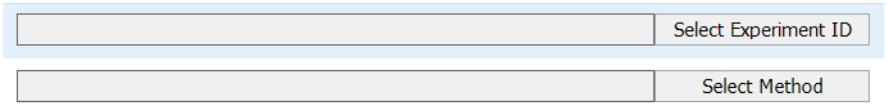
Use the Reagent Volume Calculator to automatically include excess volume, or look up the recommended value for each allowable labware type in the *AssayMAP Labware Reference Guide*, which is available in the Literature Library page of the workbench.

Step 3. Prepare the system

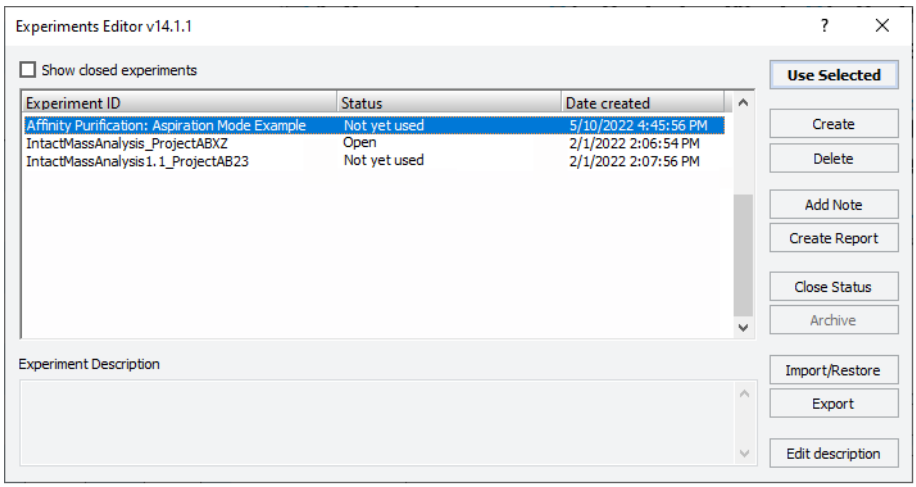
To prepare the system:

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.

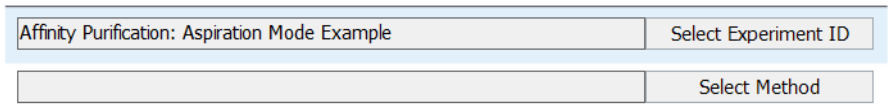
   Note: For detailed instructions, see the user guide for this utility.
4. If applicable, click **Select Experiment ID** to open the Experiments Editor.



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

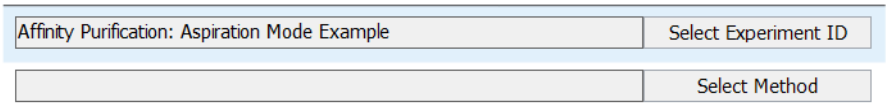
To set up the cartridges:

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.

Step 5. Run the application

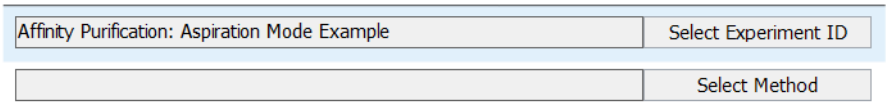
To run the application:

1 Open the **Affinity Purification: Aspiration Mode v3.0** 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 the 5 µL cartridge size. The default method file name has the 5 µL cartridge size as a prefix. This application has not yet been optimized for the 25 µL cartridges.

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 **5 µL Cartridges** from the **Number of** list.
- In the box, type the number of cartridges present in the cartridge holder at deck location 2. (Range: 8–96).

Note: Instead of specifying the number of columns, make sure you specify the number of cartridges for this application.

The position of the 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 Save Method. In the **Save File As** dialog box, type the file name and click **Save**.

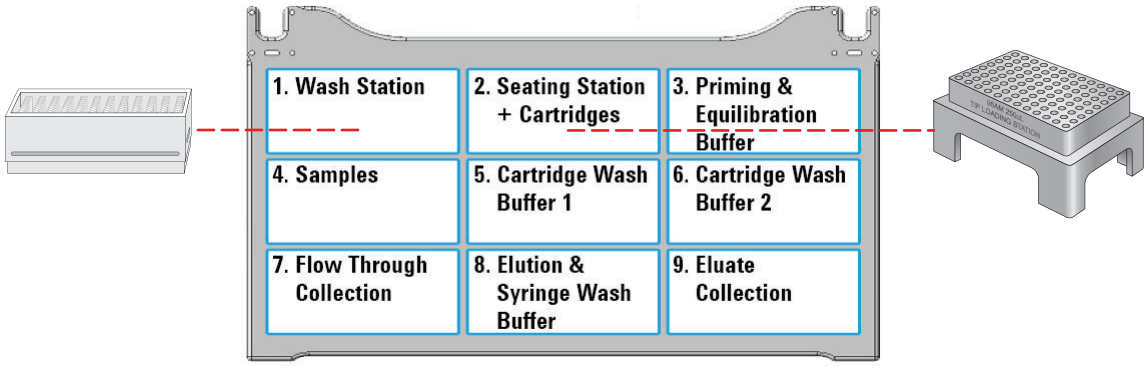
Note: Agilent recommends that you use the cartridge size (5 µL) as a prefix to the name so that you know if the method matches the cartridge size in use.

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 PCR plate is not compatible with the 25 µL cartridges at deck locations 4, 7, and 9.

6 Ensure that 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.

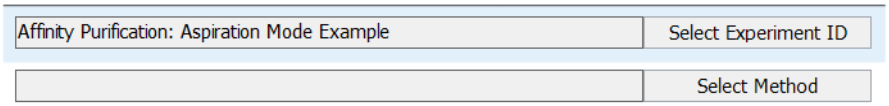
3 *Optional*. Conduct stringent washing of the syringes:

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

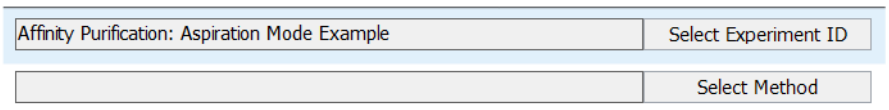
To shut down at the end of the day:

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 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.

Application Settings

The following table provides an overview of the Application Settings section in the Affinity Purification: Aspiration Mode v3.0 app.

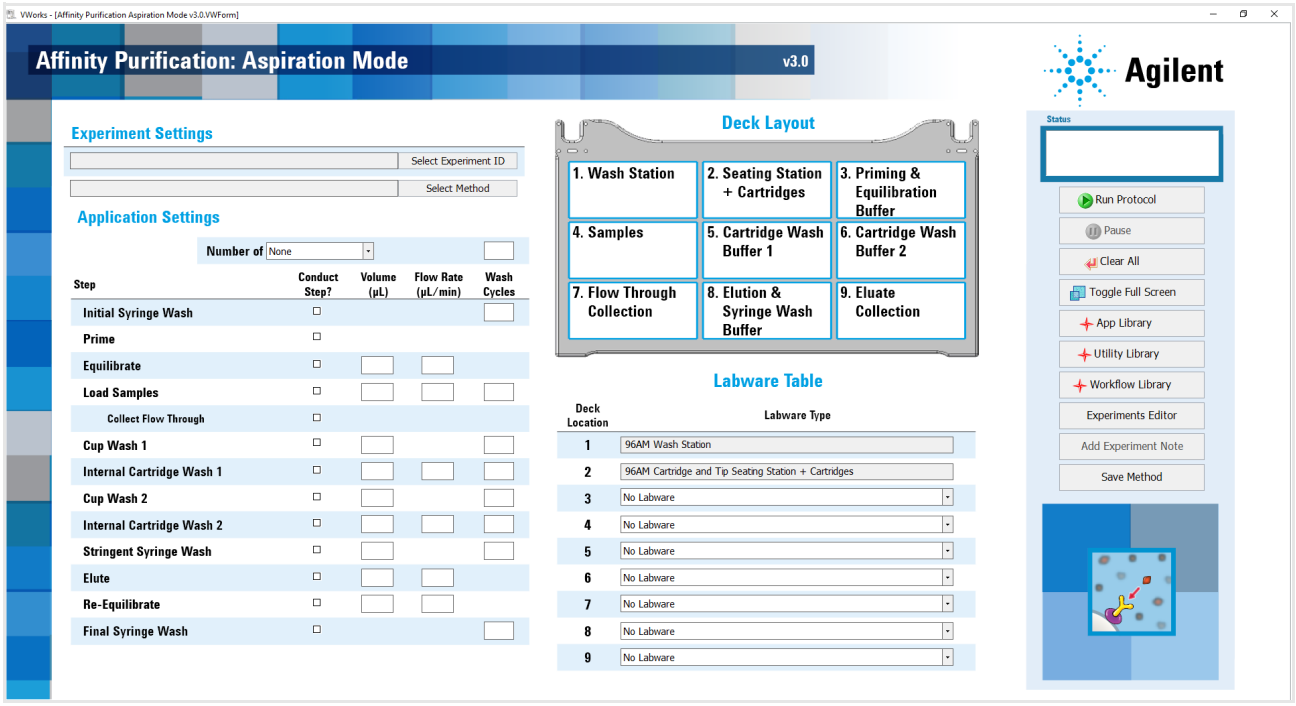


Table **Application Settings overview**

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
		Range:	–	–	0–10
Prime	Aspirates the Priming & Equilibration buffer (deck location 3) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1).	5 µL:	100	300	–
		Range:	100	300	–
Equilibrate	Dispenses the Priming & Equilibration buffer through the cartridges into the wash station (deck location 1).	5 µL:	50	10	–
		Range:	0–140	0.5–500	–

Steps*	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Load Samples	Aspirates up to 245 µL of samples (deck location 4) through the mounted cartridges into the syringes, performs an external cartridge tip wash at the wash station (deck location 1), and then aspirates a 5- µL chase of Equilibration Buffer (deck location 3). The cartridges are removed (deck location 2) and then the flow-through is dispensed into either Flow Through Collection (deck location 7) or the wash station (deck location 1). Samples >245 µL are loaded in multiple steps.	5 µL:	100	5	3
		Range:	0–1000	0.1–500	0–10
Collect Flow Through	If selected, collects the sample flow-through at the Flow Through Collection (deck location 7). If not selected, discards the sample flow-through at the wash station (deck location 1).	–	–	–	–
Cup Wash 1	Rinses the cartridge cups with Cartridge Wash Buffer 1 (deck location 5), and then discards the liquid into the wash station (deck location 1).	5 µL:	25	–	3
		Range:	0–100	–	0–10
Internal Cartridge Wash 1	Aspirates Cartridge Wash Buffer 1 (deck location 5) through the mounted cartridges, removes the cartridges from the probes, and dispenses the contents of the syringes into the wash station (deck location 1).	5 µL:	50	10	3
		Range:	0–250	0.5–500	0–10
Cup Wash 2	Rinses the cartridge cups with Cartridge Wash Buffer 2 (deck location 6) and discards the liquid into the wash station (deck location 1).	5 µL:	25	–	3
		Range:	0–100	–	0–10
Internal Cartridge Wash 2	Aspirates Cartridge Wash Buffer 2 (deck location 6) through the mounted cartridges, removes the cartridges from the probes, and dispenses the contents of the syringes into the wash station (deck location 1).	5 µL:	50	10	3
		Range:	0–250	0.5–500	0–10
Stringent Syringe Wash	Aspirates Syringe Wash Buffer (deck location 8) into the syringes, and then discards the liquid into the wash station (deck location 1).	5 µL:	50	–	2
		Range:	0–250	–	0–10
Elute	Aspirates Elution Buffer (deck location 8) into the syringes, and then dispenses the buffer through the cartridges into the Eluate Collection plate (deck location 9).	5 µL:	25	5	–
		Range:	0–250	0.1–500	–

Steps*	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Re-Equilibrate	Aspirates Equilibration Buffer (deck location 3) through the mounted cartridges into the syringes (aspirate mode), removes the cartridges, and then dispenses liquid into the wash station (deck location 1).	5 µL:	50	10	–
		Range:	0–250	0.5–500	–
Final Syringe Wash	Washes the syringes at the wash station (deck location 1).	5 µL:	–	–	3
		Range:	–	–	0–10

*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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