AssayMAP Protein Sample Prep Workbench

Protein Cleanup v2.0 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 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

Use the **Reagent Volume Calculator** for Protein Cleanup v2.0 to:

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

For in-depth assay development guidelines, see the *Protein Cleanup v2.0 User Guide* in the Literature Library of the Protein Sample Prep Workbench.

Step 2. Prepare reagent and sample 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 Run the **Cartridge Transfer** utility to set up the cartridges  .

2 Open the **Protein Cleanup v2.0** app.

3 Under **Select Method**, click ..., and then locate and select the method for the cartridge size you are using (5 or 25 µL). Click **Load** to display all the settings associated with the selected method.

Note: Protein Cleanup methods are saved in the C:\VWorks Workspace\Methods\Protein Cleanup 2.0 folder. Agilent provides a method with default settings for each cartridge size. Each default method file name has the cartridge size as a prefix.

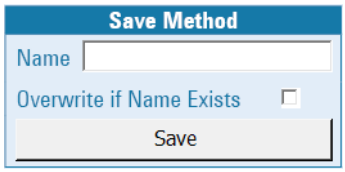
To modify the selected method, proceed to step 4. Otherwise, go to step 5.

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.

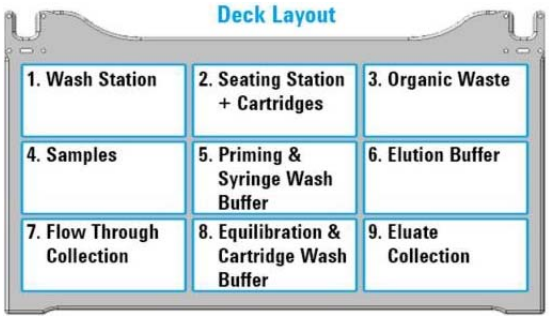
c Specify the method **Name** in the **Save Method** area, and if applicable, click **Overwrite if Name Exists**. Click **Save.**



Note: Agilent recommends using the cartridge size as a prefix for your method naming convention so that you know if the method matches the cartridge size in use.

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 Place filled reagent 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.

Step 5. Clean up after each run

To clean up after the run:

1 Remove used labware from the deck.

2 Discard the organic waste and leftover reagents appropriately.

3 *Optional*. To conduct stringent washing of the syringes, run the **Syringe Wash** utility  .

Step 6. Shut down at end of day

To shut down at the end of the day:

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

2 Remove everything from the deck except the 96AM Wash Station (deck location 1) and the 96AM Cartridge & Tip Seating Station (deck location 2), and then 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 tables provide an overview of the Application Settings section in the Protein Cleanup v2.0 app.

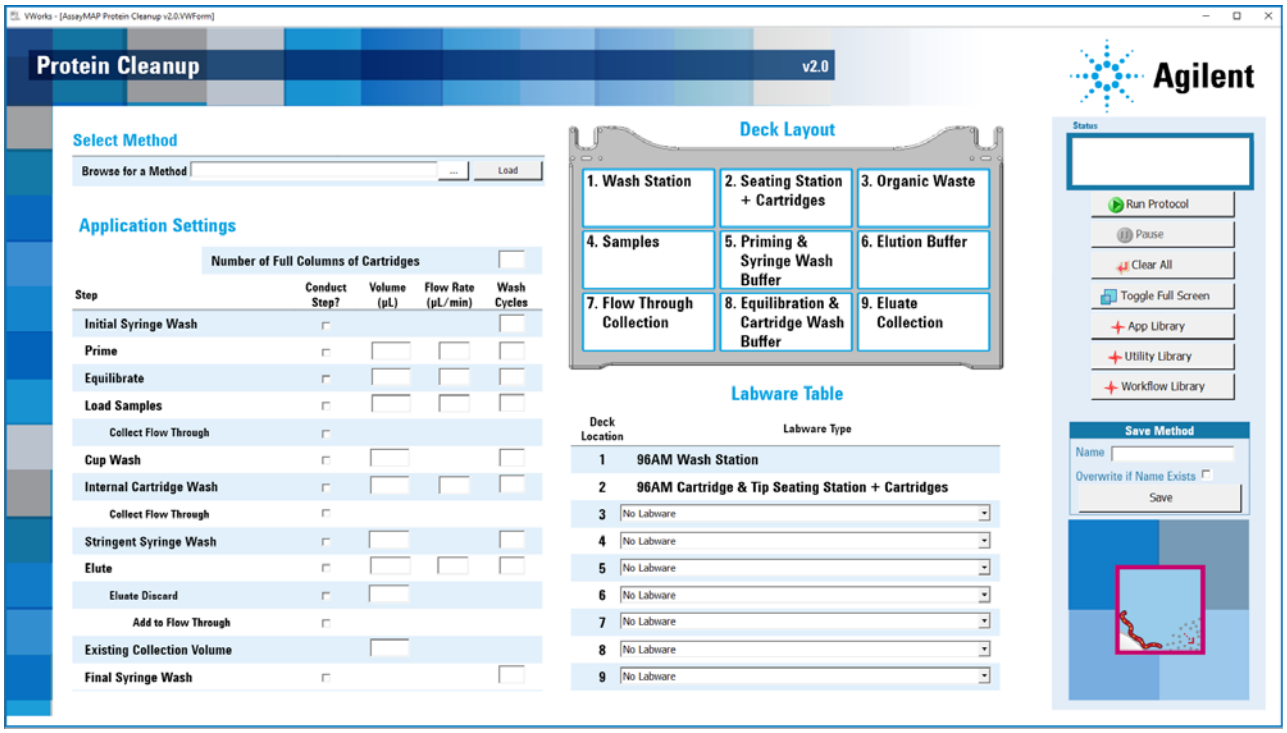


Table **Application Settings overview**

Setting	Description	Default value (range)
Number of Full Columns of Cartridges	Specifies the number of full columns of cartridges in the 96AM Cartridge & Tip Seating Station (deck location 2).	1 (1–12)

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
Prime	Aspirates Priming Buffer (deck location 5) into the syringes, and then dispenses it through the cartridges into the Organic Waste plate (deck location 3).	5 µL:	100	300	1
		25 µL:	250	300	1
		Range:	0–250	0.5–500	0–10
Equilibrate	Aspirates Equilibration Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into the Organic Waste plate (deck location 3).	5 µL:	50	10	1
		25 µL:	250	10	1
		Range:	0–250	0.5–500	0–10

Steps	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Load Samples	Aspirates samples (deck location 4) into the syringes, and then dispenses them through the cartridges into the Organic Waste plate (deck location 3) or into the Flow Through Collection plate (deck location 7).	5 µL:	100	5	3
		25 µL:	100	5	3
		Range:	0–1000	0.1–500	0–10
Collect Flow Through	If selected, collects the sample flow-through in the Flow Through Collection plate (deck location 7). If not selected, discards the sample flow-through into the Organic Waste plate (deck location 3).	–	–	–	–
Cup Wash	Rinses the cartridge cups with Cartridge Wash Buffer (deck location 8), and then discards the liquid into the Organic Waste plate (deck location 3).	5 µL:	25	–	3
		25 µL:	25	–	3
		Range:	0–100	–	1–10
Internal Cartridge Wash	Aspirates the Cartridge Wash Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into the Organic Waste plate (deck location 3) or into the Flow Through Collection plate (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 flow-through in the Flow Through Collection plate (deck location 7). If not selected, discards the Internal Cartridge Wash flow-through into the Organic Waste plate (deck location 3).	–	–	–	–
Stringent Syringe Wash	Aspirates the Syringe Wash Buffer (deck location 5) into the syringes, and then dispenses it into the Organic Waste plate (deck location 3).	5 µL:	50	–	2
		25 µL:	50	–	2
		Range:	0–250	–	1–10
Elute	Aspirates the Elution Buffer (deck location 6) into the syringes, and then dispenses it through the cartridges into the Eluate Collection plate (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 the eluate is discarded into the Organic Waste plate (deck location 3), or collected in the Flow Through Collection plate (deck location 7).	5 µL:	0	–	–
		25 µL:	0	–	–
		Range:	0–250	–	–
Add to Flow Through	If selected, collects the Eluate Discard in the Flow Through Collection plate (deck location 7). If not selected, discards the Eluate Discard into the Organic Waste plate (deck location 3).	–	–	–	–

Steps	Description	Cartridge size	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
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:	0–300	–	–
Final Syringe Wash	Washes the syringes at the wash station (deck location 1).	–	–	–	3
		–	–	–	3
		–	–	–	0–10

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