AssayMAP Protein Sample Prep Workbench

Phosphopeptide Enrichment v2.1 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 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 Phosphopeptide Enrichment v2.1 to:

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

For in-depth assay development guidelines, see the *Phosphopeptide Enrichment v2.1 User Guide* in the Literature Library of the Protein Sample Prep Workbench.

Step 2. Prepare the sample and 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.

Step 4. Run the application

To run the application:

1 Run the **Cartridge Transfer** utility to set up the cartridges  .

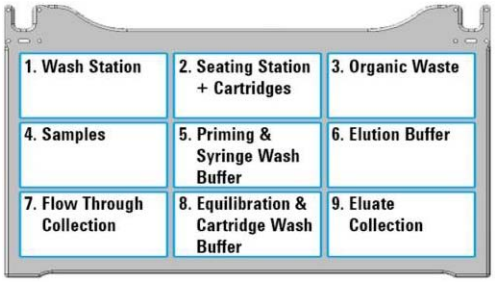
2 Open the **Phosphopeptide Enrichment v2.1** app.

3 Under **Application Settings**, select the settings appropriate for your run. For help, see "Application Settings" on page 3.

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.

4 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.

5 In the **Labware Table** of the app interface, select the labware for your run.

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 Phosphopeptide Enrichment v2.1 app.

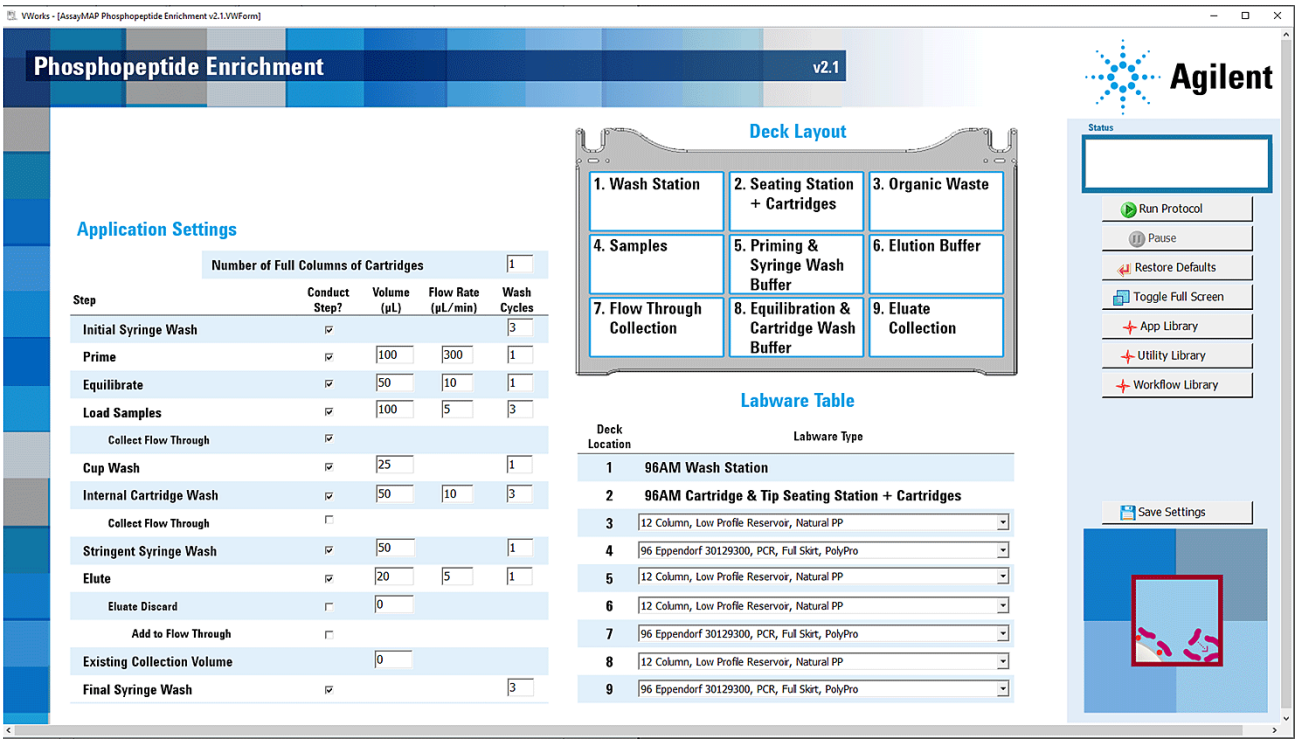


Table **Application Settings overview**

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

Steps	Description	Volume (µL)	Flow Rate (µL/min)	Wash Cycles
Initial Syringe Wash	Washes syringes at the wash station (deck location 1).	–	–	3 (0–10)
Prime	Aspirates Priming Buffer (deck location 5) into the syringes, and then dispenses it through the cartridges into the Organic Waste (deck location 3).	100 (0–250)	300 (0.5–500)	1 (0–10)
Equilibrate	Aspirates Equilibration Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into the Organic Waste (deck location 3).	50 (0–250)	10 (0.5–500)	1 (0–10)

Steps	Description	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 Flow Through Collection (deck location 7), or into the Organic Waste (deck location 3).	100 (0–1000)	5 (0.5–500)	3 (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 into the Organic Waste (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 (deck location 3).	25 (0–100)	–	1 (1–10)
Internal Cartridge Wash	Aspirates Cartridge Wash Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into the Organic Waste (deck location 3) or into the Flow Through Collection (deck location 7).	50 (0–250)	10 (0.5–500)	3 (0–10)
Collect Flow Through	If selected, collects the Internal Cartridge Wash flow-through at the Flow Through Collection (deck location 7). If not selected, discards the Internal Cartridge Wash flow-through into the Organic Waste (deck location 3).	–	–	–
Stringent Syringe Wash	Aspirates Syringe Wash Buffer (deck location 5) into the syringes, and then dispenses it into the Organic Waste (deck location 3).	50 (0–250)	–	1 (1–10)
Elute	Aspirates Elution Buffer (deck location 6) into the syringes, and then dispenses it through the cartridges into the Eluate Collection (deck location 9).	20 (0–250)	5 (0.5–500)	1 (0–10)
Eluate Discard	If selected, a specified initial volume of the Eluate will be collected at the Flow Through Collection (deck location 7), or discarded into the Organic Waste (deck location 3).	0 (0–25)	–	–
Add to Flow Through	If selected, collects the Eluate Discard at the Flow Through Collection (deck location 7). If not selected, discards the Eluate Discard into the Organic Waste (deck location 3).	–	–	–
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	Washes the syringes at the wash station (deck location 1).	–	–	3 (0–10)

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