

### AssayMAP Protein Sample Prep Workbench

## Peptide Cleanup: Aspiration Mode 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 **Peptide Cleanup: Aspiration Mode v2.0 Reagent Volume Calculator** to:

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

For in-depth assay development guidelines, see the *Peptide Cleanup: Aspiration Mode v2.0 User 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.



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.



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

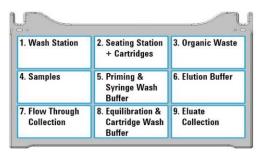


- 2 Open the **Peptide Cleanup: Aspiration Mode v2.0** app.
- **3** Under **Application Settings**, select the settings appropriate for your run. For help, see "Application Settings" on page 3.

#### 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 that is required 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

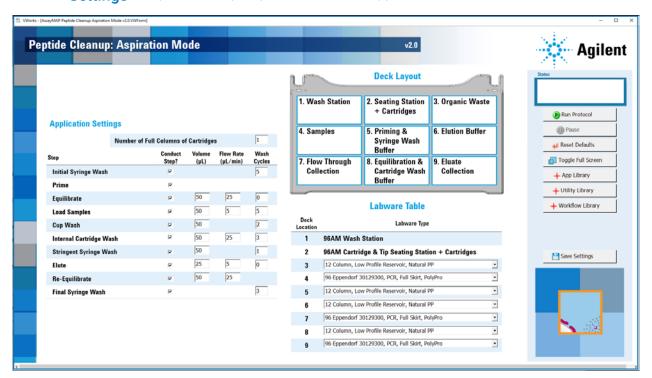
#### To shut down at the end of the day:



- 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 Peptide Cleanup: Aspiration Mode v2.0 app.



#### Table Application Settings overview

| Setting                                    | Description  | Default value (range) |
|--|--|-----------------------|
| Number of Full<br>Columns of<br>Cartridges | Specifies the number of full columns in the cartridge holder at deck location 2. | 1 (1-12)              |

| Steps                      | Description   | Volume<br>(μL) | Flow Rate<br>(µL/min) | Wash<br>Cycles |
|----------------------------|---|----------------|-----------------------|----------------|
| Initial Syringe<br>Wash    | Washes syringes at the wash station (deck location 1).  | -              | -                     | 5<br>(1–10)    |
| Prime                      | Aspirates Priming Buffer (deck location 5) into the syringes, mounts the cartridges, dispenses the buffer through the cartridges into the Organic Waste (deck location 3), and then parks the cartridges (deck location 2).   | -              | -                     | -              |
| Equilibrate                | Aspirates Equilibration Buffer (deck location 8) into the syringes, mounts the cartridges, dispenses buffer through the cartridges into the wash station (deck location 1), parks the cartridges, and then washes the syringes at the wash station.   | 50<br>(0-250)  | 25<br>(0.5-500)       | 0<br>(0-10)    |
| Load Samples               | Mounts the cartridges, aspirates the specified volume of samples (deck location 4) through the cartridges at the specified flow rate, performs an external cartridge tip wash at the wash station (deck location 1), and then aspirates a 5 $\mu$ L chase of Equilibration Buffer (deck location 8). The cartridges are parked at the tip seating station (deck location 2), and the syringes dispense their contents into the Flow Through Collection plate (deck location 7). | 50<br>(0-245)  | 5<br>(0.5–500)        | 5<br>(0-10)    |
| Cup Wash                   | Rinses the cartridge cups with Cartridge Wash Buffer (deck location 8), and then discards the liquid into the wash station (deck location 1).   | 50<br>(1–100)  | -                     | 2<br>(1–10)    |
| Internal Cartridge<br>Wash | Mounts the cartridges, aspirates Cartridge Wash Buffer (deck location 8) through the cartridges, parks the cartridges, dispenses the contents of the syringes into the wash station at an offset from the chimneys, and then washes the syringes at the wash station.   | 50<br>(0-250)  | 25<br>(0.5–500)       | 3<br>(0-10)    |
| Stringent Syringe<br>Wash  | Aspirates Syringe Wash Buffer (deck location 5) into the syringes, dispenses the buffer into Organic Waste (deck location 3), and then performs a wash at the wash station.   | 50<br>(1-250)  | -                     | 1<br>(1–10)    |
| Elute                      | Aspirates Elution Buffer (deck location 6) into the syringes, washes the probes at the wash station (deck location 1), mounts the cartridges, dispenses the buffer through the cartridges into the Eluate Collection plate (deck location 9). The cartridge tips are washed at the wash station, and then the cartridges are parked (deck location 2). The syringes mix the eluate, and then the syringes are washed at the wash station.                                       | 25<br>(0-250)  | 5<br>(0.5–500)        | 0 (0-10)       |
| Re-Equilibrate             | Aspirates Equilibration Buffer (deck location 8) into the syringes (aspirate mode), mounts the cartridges, dispenses the buffer into the wash station (deck location 1), and then parks the cartridges.   | 50<br>(0-250)  | 25<br>(0.5–500)       |                |
| Final Syringe Wash         | Washes the syringes at the wash station (deck location 1).  | _              | _                     | 3<br>(1–10)    |

Contacting Agilent Technologies

Web: https://www.agilent.com

Contact page: https://www.agilent.com/en/contact-us/page

Documentation feedback: documentation.automation@agilent.com