AssayMAP Protein Sample Prep Workbench

Evosep - 96AM Workflow Quick Start Guide

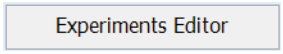
This guide is for users who have been trained in the proper use of the AssayMAP Bravo Platform and understand the safety guidelines in the *Bravo Platform Safety and Installation Guide*. The procedures in this guide require the Protein Sample Prep Workbench.

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

  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

The Agilent Evosep 96AM Workflow application automates the pipetting steps of the Evosep loading protocol for Evotip Pure. Refer to the protocol guide at www.evosep.com for a list of the required solutions and equipment.

The following table provides a complete list of labware and the corresponding deck locations on the AssayMAP Bravo Platform.

CAUTION

Use only the labware specified for each deck location. Using different labware or placing labware at unapproved deck locations can cause a collision resulting in equipment damage.

Table Labware and corresponding AssayMAP Bravo deck locations

Labware	Manufacturer part number*	Deck location options
250-µL pipette tips	Agilent 19477-002	5, 6**
96 Abgene PCR	Fisher Scientific AB-0800	4
96 Eppendorf 30129300, PCR, Full Skirt, PolyPro	Eppendorf 30129300	4
96 Bio-Rad PCR, Hard-Shell, Low-Profile, Full Skirt	Bio-Rad HSP-9611	4
96 Greiner 652270, PCR, Full Skirt, PolyPro	Greiner 652270	4
Reservoir, Seahorse 201254-100, PP, 1 Well, pyramid bottom	Agilent 201254-100	3, 9
Evotip Holder with Evotips	*Holder:* Evosep EV1142 *Evotips:* Evosep EV2001	7
Equilibration Holder + Reservoir, Seahorse 201254-100	*Holder:* Evosep EV1142 *Reservoir:* Agilent 201254-100	8

*For dimensionally equivalent alternatives and other labware details, see the *Labware Reference Guide* in the Literature Library page of the Protein Sample Prep Workbench.

**If less that 12 full columns are used in the sample plate, the Tip Setup protocol is required to prepare the pipette tip layout. The Tip Setup protocol uses empty pipette tip boxes at locations 8 and 9.

Step 2. Prepare reagent plates

To minimize evaporation, fill the labware immediately before run time or keep them covered until you run the protocol. See the Evosep protocol guide at www.evosep.com for the reagent volume requirements.

CAUTION

A small reagent volume excess is required in all labware types to ensure proper volume transfer.

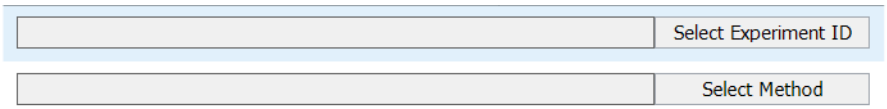
Look up the recommended volume excess required for each allowable labware type in the *AssayMAP Labware Reference Guide*, which is available in the Literature Library page of the workbench.

Step 3. Prepare the system

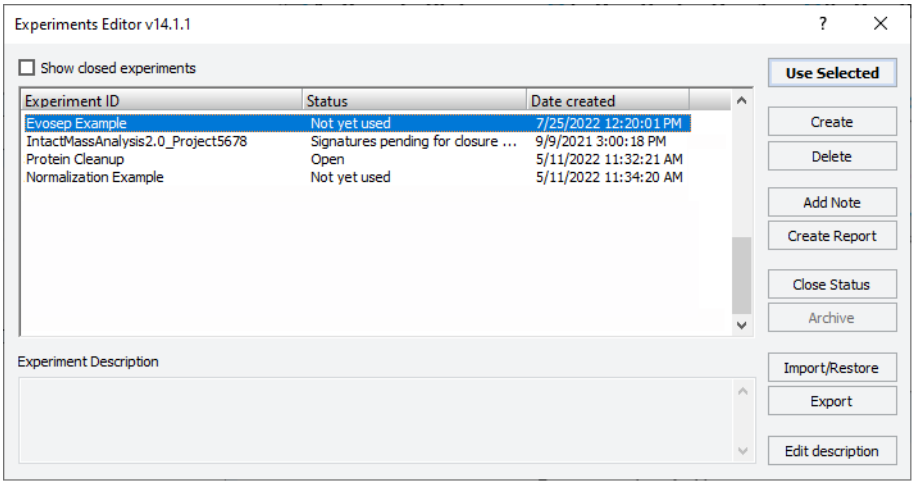
To prepare the system:

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.

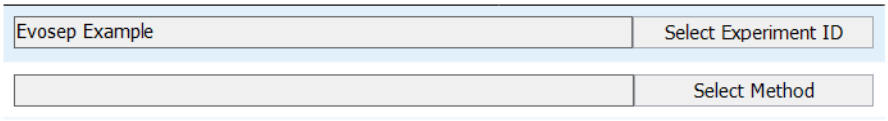
   Note: For detailed instructions, see the user guide for this utility.
4. If applicable, click **Select Experiment ID** to open the Experiments Editor.



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.

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.**

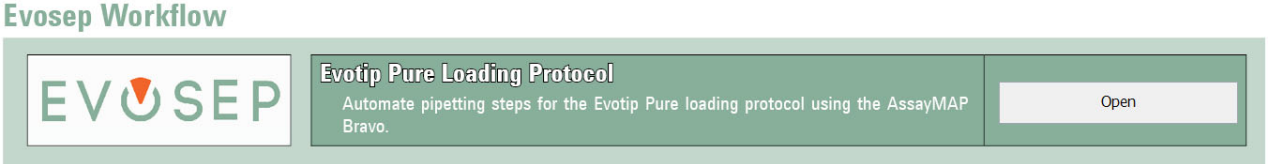
9 During the Startup protocol, verify that all the wash station chimneys have liquid flowing through them. If liquid is not flowing through the chimneys, see the *96 Channel Wash Station Maintenance Guide* for troubleshooting guidelines.

Step 4. Specify app settings

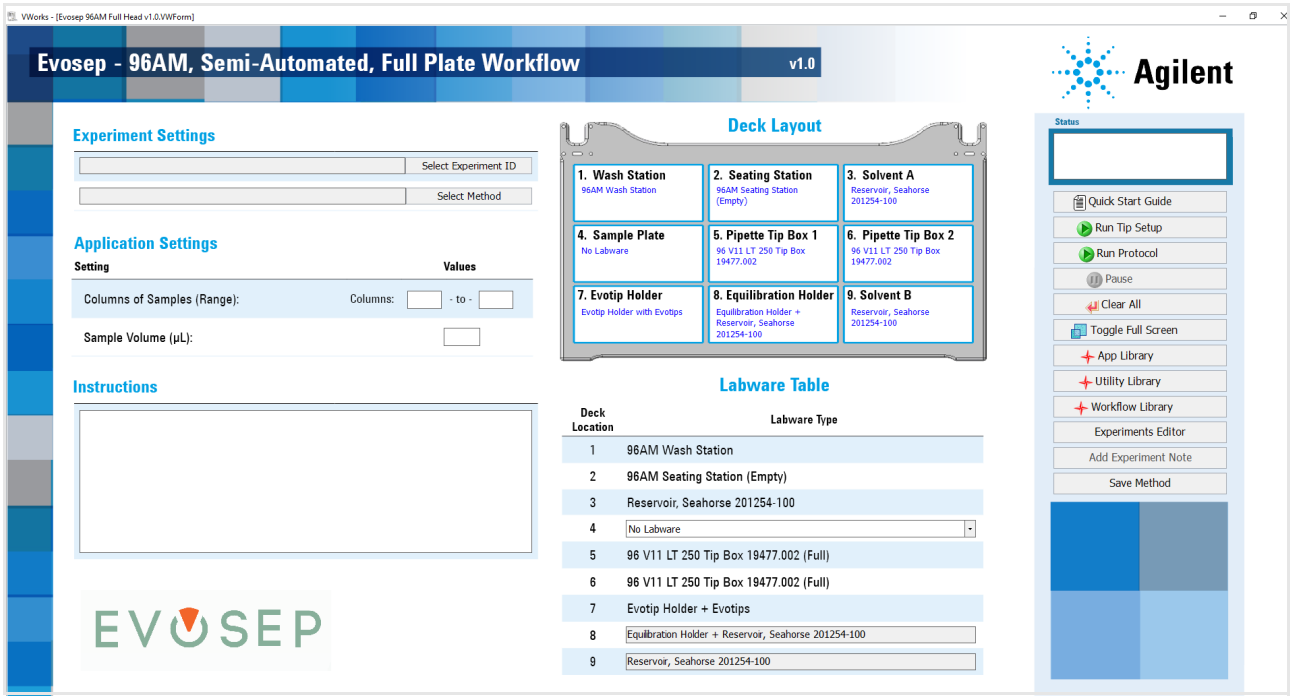
To specify the settings for the Evosep 96AM Workflow app:

1 Open the **Workflow Library**.

2 Locate the **Evosep 96AM Workflow**, and then click **Open**.



The Evosep 96AM, Semi-Automated Full-Plate Workflow application opens.



3 If applicable, click **Select Experiment ID** to open the Experiments Editor.

4 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**.

5 In the form, click **Select Method** to select and load the method for this application.

To modify the selected method, proceed to step 6.

Otherwise, go to "Step 5. Run the Tip Setup protocol" on page 5.

6 To create or modify a method:

VWorks Plus. Administrator or technician privileges are required to create or modify methods.

a In the **Columns of Samples (Range)**, specify the range of columns in the Sample Plate, for example, columns 1 to 12 for samples in all 96 wells.

b In the **Sample Volume (µL)** field, type the value for the volume in each well of the Sample Plate.

Range: 5–100

Default: 20

If you use a value other than the default, refer to the Evosep protocol guide at www.evosep.com.

c In the **Labware Table** of the form, select the labware for the Sample Plate at deck location 4.

d To save the method, click Save Method. In the **Save File As** dialog box, type the file name and click **Save**.

VWorks Plus. You must save the method before you can run it.

Step 5. Run the Tip Setup protocol

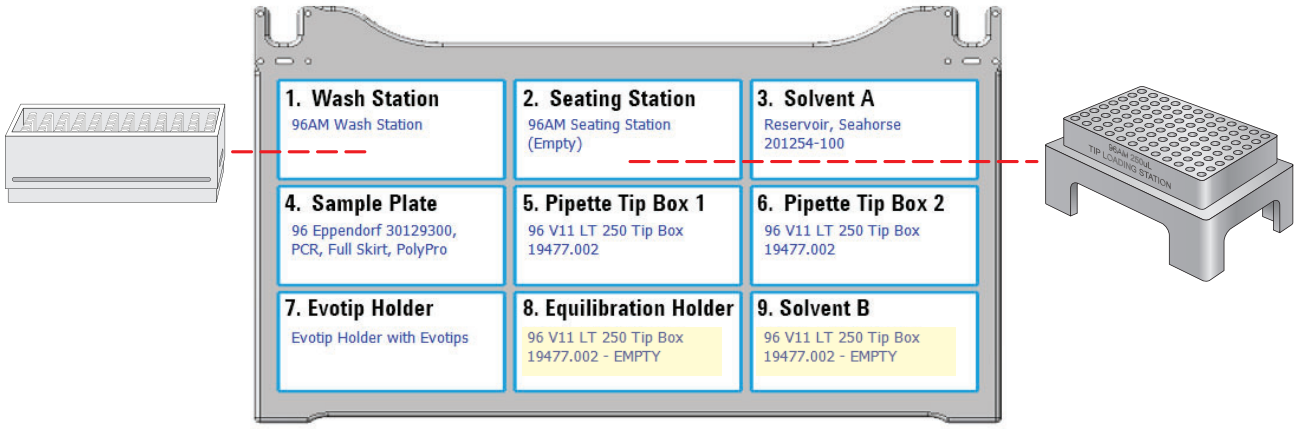
If running fewer than 12 columns in the sample plate, perform the following procedure to set up the pipette tips.

Tip Setup is not required when running 12 columns in the sample plate. In this case, skip to "Step 6. Run the application" on page 6.

To run the Tip Setup protocol:

1. Click Run Tip Setup to start the run.
2. Follow the **Instructions** that appear on the form.

IMPORTANT Ensure that empty Agilent 250 µL pipette tip boxes are at deck locations 8 and 9, as described in the form Instructions.



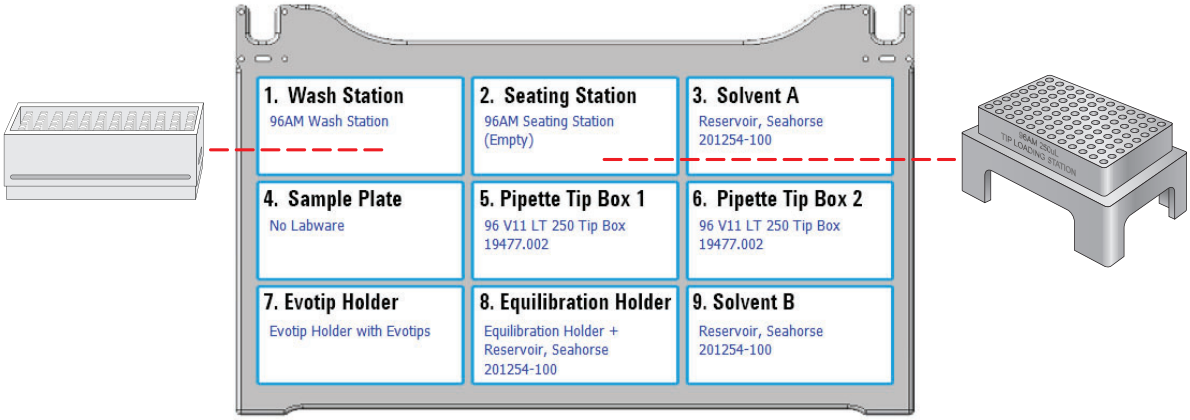
WARNING **The probes of the Bravo 96AM Head are sharp and can scratch you if they brush across your hand. A probe scratch can expose you to any contaminants remaining on the probes. Be careful to avoid touching the probes.**

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.**

Step 6. Run the application

To run the application:

1 Ensure that the accessories, filled reagent plates, pipette tips, Evotip Holder, and Equilibration Holder + reservoir are at the assigned deck locations, as shown in the **Deck Layout** image of the form.



2 Click Run Protocol to start the run.

3 Follow the **Instructions** that appear on the form.

Step 7. Clean up after each run

To clean up after the run:

1 Remove used labware from the deck.

2 Discard leftover reagents appropriately.

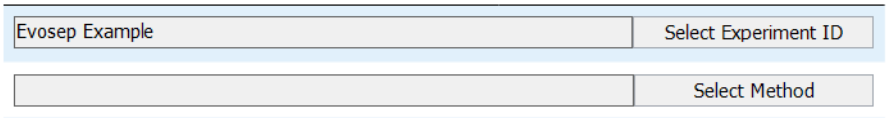
3 *Optional*. Conduct stringent washing of the syringes:

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.

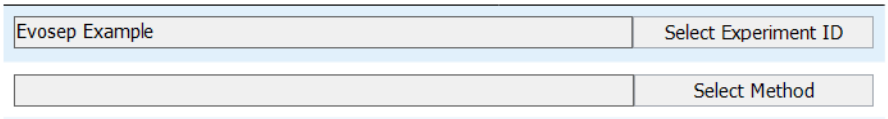
Step 8. Shut down at end of day

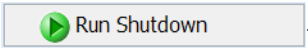
To shut down at the end of the day:

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 

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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.

Contacting Agilent Technologies

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