AssayMAP Protein Sample Prep Workbench

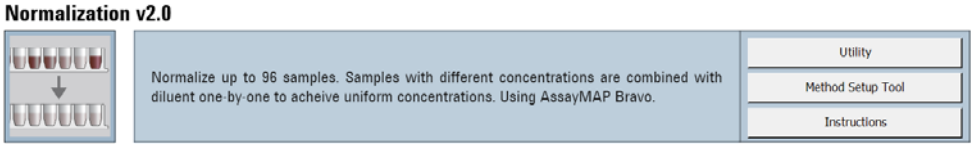
Normalization v2.0 Quick Start Guide

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 the Normalization method

Open the **Normalization Method Setup Tool v2.0** and follow the instructions on the screen to design and save a normalization method.

To open the Normalization Method Setup Tool, locate the **Normalization v2.0** banner in the **Utility Library**, and then click **Method Setup Tool**. Follow the instructions on the screen to design and save a method file.



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

CAUTION

A small sample and reagent volume excess is required in all labware types to ensure proper volume transfer. The Normalization Method Setup Tool automatically indicates the amount of excess volume recommended per plate type but this volume can be changed by the user.

Step 2. Prepare Sample and Diluent plates

Prepare the Sample plate to match the initial sample volumes and well positions specified in the method that you created in the Normalization Method Setup Tool. Prepare the Diluent plate by putting the volume calculated by the Normalization Method Setup Tool in position A12, as this is where all the diluent will be aspirated.

IMPORTANT

To minimize evaporation, fill the Sample and Diluent plates immediately before run time or keep them covered until you run the protocol.

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 utility

To run the Normalization utility:

1 Open the **Normalization** utility.

2 Under **Select and Load a Normalization Method**, click ... and select the method. The default method storage location is C:/VWorks Workspace/Methods/AM Normalization Utility v2.0.

3 Click **Display Bravo Layout** to display the Method Loaded and the Deck Layout information.

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 Ensure that the following items are securely in place at their respective AssayMAP Bravo deck locations:

- Bravo Plate Riser at deck locations 2 and 6.
- A tip box full of fresh 250-µL pipette tips at deck location 3.
- The empty 96AM Cartridge & Tip Seating Station at deck location 5.

CAUTION

To prevent a potential collision, ensure that no thermal plate insert is on the Peltier Thermal Station installed at deck location 4.

5 Place the filled reagent plates at the assigned deck locations, as shown in the **Deck Layout** of the form.

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.

6 Ensure that all the labware on the deck exactly matches the **Deck Layout** in the form.

CAUTION

Incorrect labware selections can cause a hardware collision, resulting in equipment damage. Ensure that the selections in the method exactly match the physical labware present on the Bravo deck.

7 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 used pipette tips from the tip box at deck location 3.

3 Transfer the unused pipette tips from the 96AM Cartridge & Tip Seating Station at deck location 5 to unused locations in the tip box.

4 Remove the Bravo Plate Risers from deck locations 2 and 6.

5 Discard any leftover reagents appropriately.

6 *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).

3 Place the 96AM Cartridge & Tip Seating Station at deck location 2, and then click **Run Shutdown**.

4 After the Shutdown protocol has completed, turn off the power at the AssayMAP Bravo Platform and the accessories.

5 Close the Protein Sample Prep Workbench software.

Utility overview

The following figure shows the utility interface, and the following table provides an overview of the basic movements of the AssayMAP Bravo Platform during the Normalization protocol.

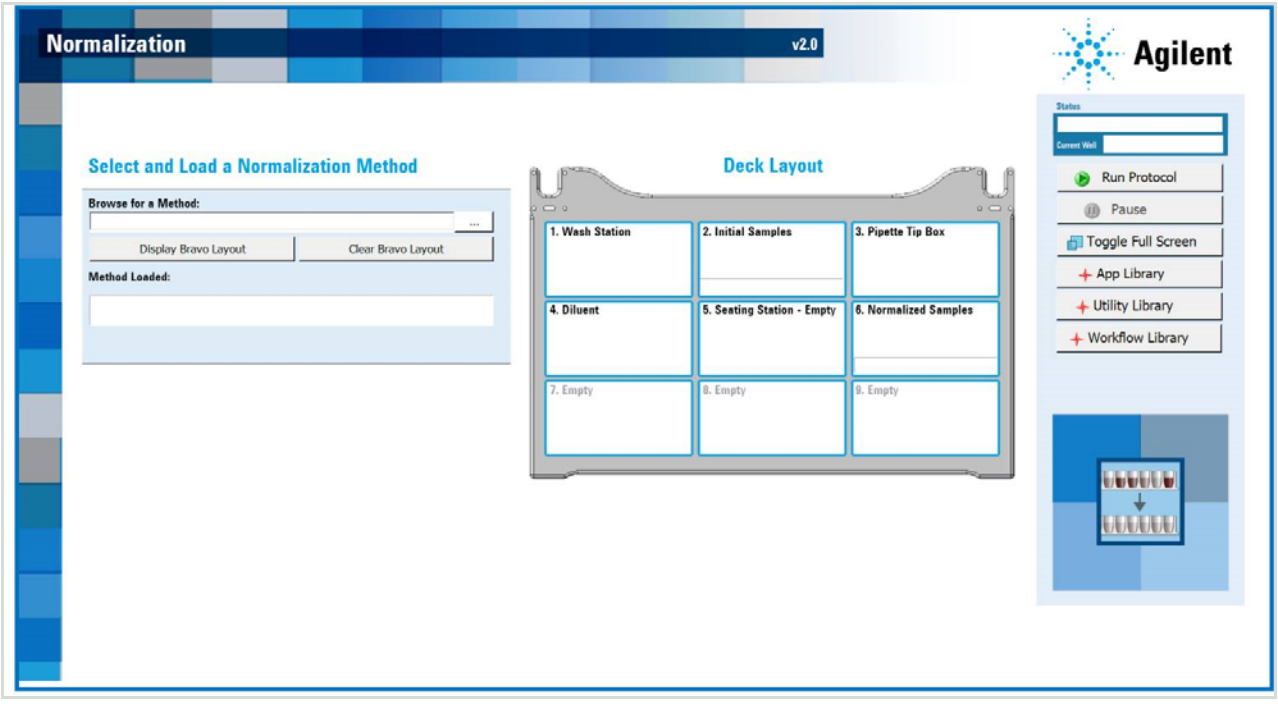


Table **Automation movements during the protocol**

Protocol process	Process name	Process description
1	Syringe Wash	Performs 1 external syringe wash at the wash station (deck location 1).
2	Syringe Drying	Performs 4 syringe aspirate-and-dispense cycles above the wash station (deck location 1) to cycle air in and out of the syringes. The syringes move over the chimneys after each cycle to remove any droplets that were pushed out of the syringes during the cycle.
3	Initial Tip Transfer	Transfers all 96 250-µL pipette tips from the tip box (deck location 3) to the 96AM Cartridge & Tip Seating Station (deck location 5).
4	Single Tip Pickup	Picks up the next available individual pipette tip from the 96AM Cartridge & Tip Seating Station (deck location 5) using probe A12 of the Bravo 96AM Head.
5	Diluent Transfer	Aspirates diluent (deck location 4, well A12) into the pipette tip, and then dispenses the diluent into a specific well in the normalized plate (deck location 6).
6	Sample Transfer	Aspirates sample (deck location 2) into the pipette tip, and then dispenses the sample into the same well in the normalized plate (deck location 6) that was used for the Diluent Transfer process.
7	Single Tip Eject	Ejects the used pipette tip into the tip box (deck location 3). The tip box well location matches the well location of the normalized sample that the pipette tip was used to prepare.
8	Additional Transfers	Repeats processes 2 through 5 for every sample in the sample plate (deck location 2).
9	Used Tip Pickup	Presses on all the used pipette tips from the tip box (deck location 3).
10	Mixing	Mixes all the samples in the normalized plate (deck location 6).
11	Final Tip Ejection	Ejects the used pipette tips into the tip box (deck location 3).

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