

# AssayMAP Protein Sample Prep Workbench

## Normalization v3.0 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. You can find more detailed instructions by going to the Literature Library in 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

**Experiments Editor**

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 the Normalization method

*VWorks Plus only.* Administrator or technician privileges are required to create or modify methods. In addition, you must save the method before you can run it.

#### To open the Normalization Method Setup Tool

In the **Utility Library**, locate **Normalization v3.0**, and then click **Method Setup Tool**.

#### Normalization v3.0



Normalize up to 96 samples. Samples with different concentrations are combined with diluent one-by-one to achieve uniform concentrations. Using AssayMAP Bravo.

Utility

Method Setup Tool

Instructions

The Method Setup Tool has 13 distinct steps. The following figure and table provide an overview of the steps. For in-depth guidelines, see the [Normalization v3.0 User Guide](#) in the Literature Library of the Protein Sample Prep Workbench.

## Step 1. Design the Normalization method

Normalization: Method Setup Tool

v3.0

**Instructions:** Complete Steps 1 - 12 in the worksheet below. Cells highlighted in green are editable and require user input. Clear All

**Step 1) Import Concentrations:**  
Click the button to the right and follow the instructions in the popup screen to import sample concentration data.

**Step 2) Define Concentration Units:**

mg/mL

mg/mL

**Initial Sample Concentrations**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 3) Select Initial Sample Plate:** In the dropdown list, select the type of labware that will initially contain the samples.

Sample Plate

96 AbGene 1127, 1mL Deep Well, Square Well, Bot

Unusable Volume (µl per well)

30

**Step 4) Enter Initial Sample Volume:** Enter the initial sample volume and then click "Select Wells to Fill" to auto-calculate Usable Sample Volumes, in the table below.

(µl)

(µl)

**Usable Sample Volumes**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 5) Enter the Target Concentration:** Enter the target concentration for normalized samples and click "Select Wells to Fill" to auto-populate the table below.

mg/mL

mg/mL

**Target Sample Concentrations**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 6) Enter the Target Volume:** Enter the target volume from normalized samples and click "Select Wells to Fill" to auto-populate the table below.

(µl)

(µl)

**Target Final Volumes**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 7) Calculate Normalization Volume:** Click the button to the right to calculate sample and diluent combination volumes in the two tables below. Red cells represent calculated exceptions.

**Diluent Volumes to Use**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 8) Manage Calculation Exceptions:** Click "Check Exceptions" to address all red cells. Follow the instructions in the popup screen to resolve calculation issues. Final values are in a second set of tables below.

**Sample Volumes to Use**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 9) Define Remaining Labware Types:** From the dropdown lists select labware types for the Diluent Reservoir and Normalized Plate.

Normalized Plate

96 Greiner 675801, Half Area, Flat Bottom, UV Star

Maximum Volume (µl per well or channel)

180

**Step 10) Select the Number of Mix Cycles:** Use the dropdown list to the right to select the number of mix cycles used to mix normalized samples at the end of the run.

mix cycles

10

**Final Diluent Volumes**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Step 11) Input Evaporation Correction Factor**

%

%

**Step 12) Check Volume Required for Diluent Reservoir below and Adjust if Necessary**

**Step 13) Create a Normalization Method File:** Click the button to the right to save a method file that can be used with the Normalization v3.0 App to conduct the normalization procedure described by the tables below.

**Final Sample Volumes**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Diluent required for Diluent Reservoir**

Sum of Volumes from Final Diluent Table: (ml) 0.00

Specify Excess Volume: (ml)

Total to fill Reservoir--> 0.00

The table below contains calculated concentrations based on selections at step 8 if there are exceptions.

**Expected Concentrations after Normalization**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

**Table** Overview of steps in Method Setup Tool

Step	Description	
1	Import concentrations	Click <b>Import Sample Concentrations</b> , and then import the values for the known concentrations in your sample plate from a file (.xls, .xlsx, .csv, or .txt) or enter the values manually.
2	Define Concentration Units	Enter the concentration units in the green box. <i>Note:</i> This input is only a reminder. It has no impact on the subsequent calculations.
3	Select Initial Sample Plate	Click the green <b>Sample Plate</b> box, and then select the labware from the list. The setup tool uses the value that appears in the <b>Unusable Volume</b> box (also known as <i>dead</i> volume) to calculate the usable sample volume. You can change the unusable volume manually, if desired, as the default value is a conservative estimate.
4	Enter Initial Sample Volume	Type the initial sample volume in the green ( <b>μL</b> ) box, and then select the array of wells for which this value is correct in the <b>Usable Sample Volumes</b> plate grid. The setup tool automatically enters the usable sample volume (that is, the initial sample volume minus the unusable volume) in the <b>Usable Sample Volumes</b> plate grid. Repeat step 4 until you have specified the volume for all the wells in the Sample plate that contain a sample.
5	Enter the Target Concentrations	Type the target concentration in the green box, click <b>Select Wells to Fill</b> , and then select the array of wells to be filled in the <b>Target Sample Concentrations</b> plate grid. Repeat step 5 until you have specified the concentrations for all the wells.
6	Enter the Target Volume	Type a value in the green ( <b>μL</b> ) box that you want to apply to multiple wells of the microplate, and then click <b>Select Wells to Fill</b> . Select the array of wells to be filled in the <b>Target Final Volumes</b> plate grid. Repeat step 6 until you have specified the volumes for all the wells.
7	Calculate Normalization Volumes	Click <b>Calculate Volumes</b> . The calculated volumes (μL) display in the <b>Diluent Volumes to Use</b> and the <b>Sample Volumes to Use</b> plate grids.
8	Manage Calculation Exceptions	Click <b>Check Exceptions</b> . In the <b>Manage Exceptions</b> dialog box, select the option that is appropriate for each sample that is flagged as problematic. The setup tool wizard guides you through the process.
9	Define Remaining Labware Types	Click the green <b>Normalized Plate</b> box, and then select the labware from the list. The <b>Maximum Volume (μL per well or channel)</b> for the selected plate is automatically displayed. <i>Note:</i> The final normalized sample volume must be equal to or less than the well volume of the selected plate type, or the Maximum Volume (μL per well or channel) cell will turn red. Click the green <b>Diluent Reservoir</b> box, and then select the labware from the list.
10	Select the Number of Mix Cycles	Click the green <b>mix cycles</b> box, and then select the value from the list. <i>Note:</i> The number of mix cycles is dependent on the volume, viscosity, and size of molecules in the solution being mixed. See the user guide for advice about the number of mix cycles when the volume is greater than approximately 200 μL.

## Step 2. Prepare sample and diluent plates

Step	Description	
11	Input Evaporation Correction Factor	Type a value in the green (%) box for the correction factor, and then click <b>Apply Correction Factor</b> . The setup tool automatically adjusts the values in the Final Diluent Volumes and Final Sample Volumes plate grids.  See the user guide for details on how to determine this value. This is an optional input.
12	Check Volume Required for Diluent Reservoir	Review the <b>Diluent required for Diluent Reservoir</b> area at the bottom of the worksheet. If a red highlight appears on the <b>Total to fill Reservoir</b> value, the diluent volume plus required overage is more than the selected labware can hold. In this case, select a different labware option for the <b>Diluent Reservoir</b> in step 9.  Make a note of the <b>Total to fill Reservoir</b> volume. You will use this value to prepare the diluent reservoir for the Normalization run. This volume includes the required overage.
13	Create a Normalization Method File	Click <b>Create Method File</b> . After you save the method, the Method Setup Tool closes.

### 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 in step 3, but this volume can be changed by the user.

## Step 2. Prepare sample and diluent plates

Prepare the sample and diluent plates to match the volumes and well positions specified in the method that you created in the Normalization Method Setup Tool. You can view the method preparation instructions in the Normalization utility when you select the method. Alternatively, you can export the method and view it in Microsoft Excel. See the [Normalization v3.0 User Guide](#) for details.

Ensure that the diluent plate volume is accessible from plate position A12, as this is the position where all the diluent will be aspirated.

To minimize evaporation, fill the labware immediately before run time or keep them covered until you run the protocol.

## Step 3. Prepare the system

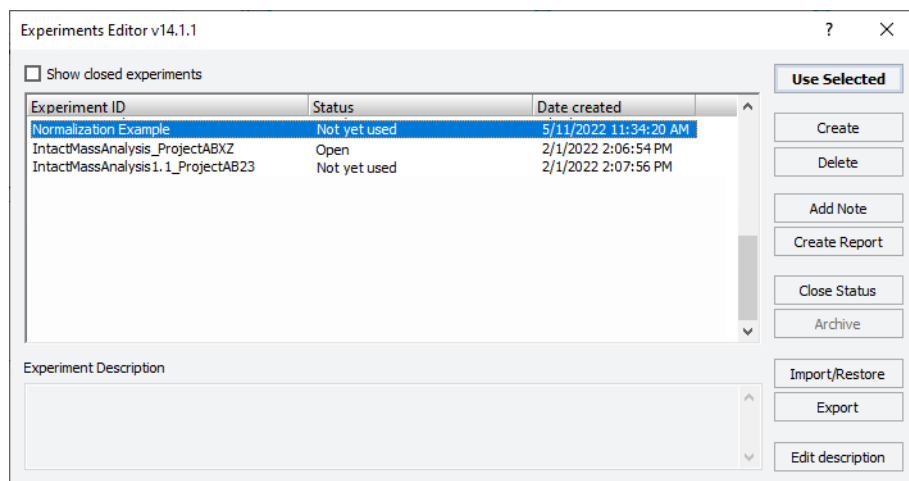


### To prepare the system:

- 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 **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.


<input type="text"/>	Select Experiment ID
<input type="text"/>	Select Method

- 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.

Normalization Example	Select Experiment ID
	Select Method

- 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. Run the utility



**To run the Normalization utility:**

- 1 Open the **Normalization** 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**.

Normalization Example	Select Experiment ID
	Select Method

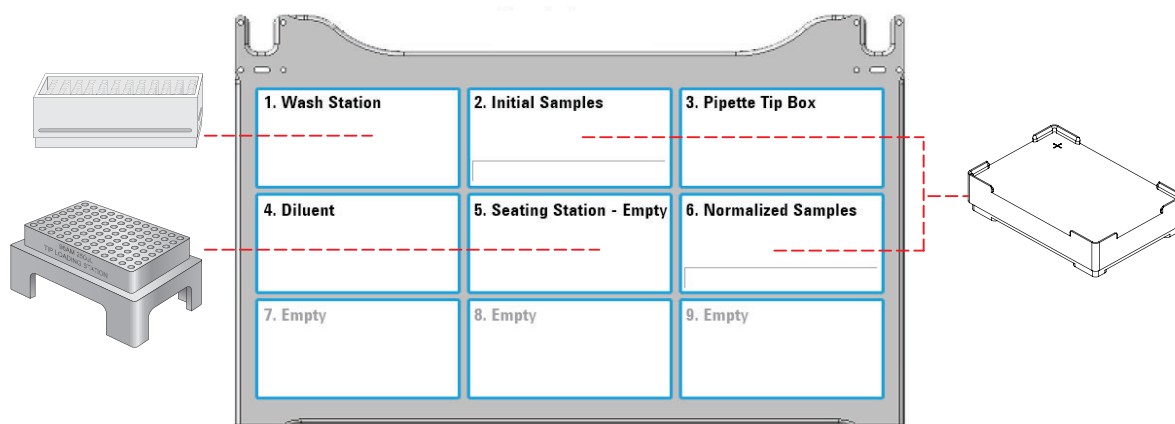
- 4 In the form, click **Select Method** to select and load the method for this utility.

## 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 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.
- The empty 96AM Cartridge & Tip Seating Station at deck location 5.



## CAUTION

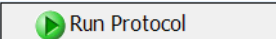
To prevent a potential collision, ensure that no thermal plate insert is on the Peltier Thermal Station installed at deck location 4.

6 Place a tip box full of fresh 250- $\mu$ L pipette tips at deck location 3, and place the filled reagent plates at the assigned deck locations, as shown in the **Deck Layout** in the form.

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

## CAUTION


Incorrect labware selections and improperly seated labware can cause hardware collisions, resulting in equipment damage. Ensure that the selections in the method 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.

7 Click  to start the run.


To monitor the progress of the run, check the **Status** box in the upper right corner of the form.

## Step 5. 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**.

Normalization Example	Select Experiment ID
	Select Method

- 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  to start the run.

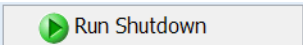
## Step 6. Shut down at end of day



### 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**.

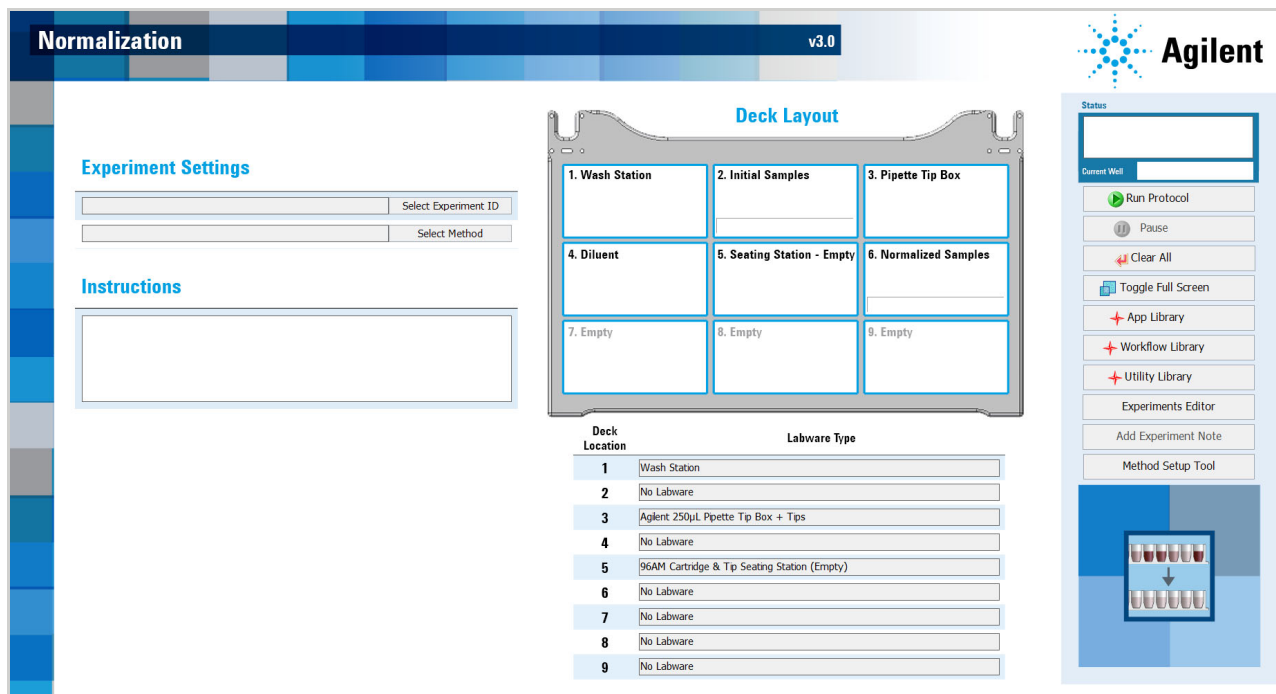
Normalization Example	Select Experiment ID
	Select Method

- 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 .
- 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.

## Utility overview

The following table summarizes 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	Washes the external part of the syringe 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) starting at position H1 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).  <i>Note:</i> If the volume is more than 140 µL this transfer will occur in multiple cycles.
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.  <i>Note:</i> If the volume is more than 140 µL this transfer will occur in multiple cycles.



Protocol process*	Process name	Process description
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 3 through 7 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). <i>Note:</i> See the user guide for advice about the number of mix cycles for volumes above 200 µL or highly viscous solutions.
11	Final Tip Ejection	Ejects the used pipette tips into the tip box (deck location 3).
*For details on conditions that can affect evaporation, correcting for loss due to evaporation, and pipetting accuracy and liquid classes, see the <i>Assay development guidelines</i> topic in the user guide.		

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