AssayMAP Protein Sample Prep Workbench

In-Solution Digestion: Multi-Plate v2.0 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 your run

Use the **In-Solution Digestion: Multi-Plate Reagent Volume Calculator** to:

- Determine reagent concentration and volume preparation requirements.
- Ensure labware selections are consistent with the volume requirements.
- Determine one of the following:
  - Volume per well required for the Master Reagent plate that is used for the Automated Plate Setup.
  - Plate setup and volume per well required for manual plate setup.

For in-depth assay development guidelines, see the *In-Solution Digestion: Multi-Plate User Guide* in the Literature Library of the Protein Sample Prep Workbench.

Step 2. Prepare the reagents

The In-Solution Digestion protocol requires a stack of five 96-well Greiner 650207 U-Bottom plates at deck location 2, regardless of the number of sample plates being used.

IMPORTANT The automated Bravo plate stacking is required to create a perfectly aligned stack, even if you do not run all the steps or if you have fewer than four sample plates.

To prepare the plates for stacking:

1. Label one plate to be used as a lid for the Protease plate, and label four plates to be used as lids for the sample plates. The four plates to be used for sample plate lids will also function as Syringe Wash Buffer plates.
2. Fill each Syringe Wash Buffer plate with 300 µL per well of buffer, such that the filled columns match the columns of samples in the sample plates.

   You may use manual pipettes for this task, or you may use the Reagent Transfer utility, Reagent Aliquot utility, or the Single Liquid Addition utility. Before using a utility, the system must be prepared in Step 3. Prepare the system.

To set up the reagent plates:

Use one of the following methods to set up the reagent plates for the In-Solution Digestion: Multi-Plate app:

- *Automated Reagent Plate Setup*. The Master Reagent plate should be prepared at this stage. In the **Reagent Volume Calculator**, display the **Automated Plate Setup** worksheet. Pipette the designated volumes of Protease, Alkylant, and Denaturation Mixtures into the assigned columns of the Master Reagent plate, as shown in the Master Reagent Plate area of the worksheet.

  Note: The Diluent plate is prepared manually because of the large volumes required.
- *Manual Reagent Plate Setup*. In the **Reagent Volume Calculator**, display the **Manual Plate Setup** worksheet. Use manual pipettes to prepare reagent plates for the Protease, Alkylant, Denaturation, and Diluent reagents based on their respective plate layouts.

  If you manually prepare your reagent plates, you will skip Step 4. Run Reagent Plate Setup and Step 5. Clean up after Reagent Plate Setup.

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.**

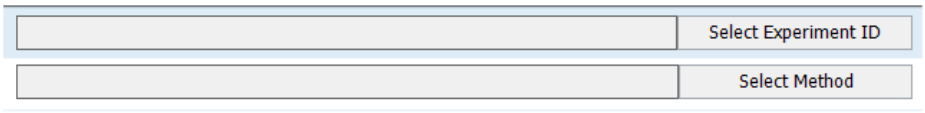
Use the Reagent Volume Calculator to automatically include excess volume, or look up the recommended value for each allowable labware type in the *AssayMAP Labware Reference Guide*, which is available in the Literature Library page of the workbench.

Step 3. Prepare the system

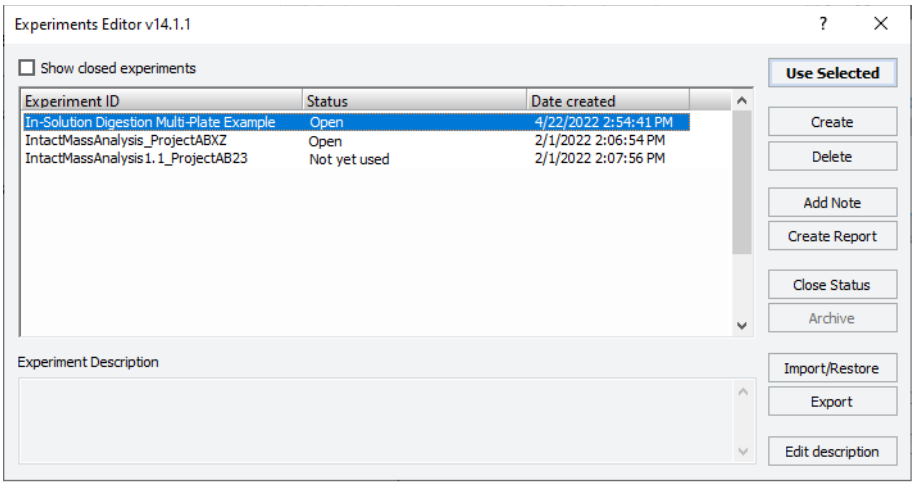
To prepare the system:

1. Check the levels of the wash station source and waste carboys. 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 

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 Reagent Plate Setup

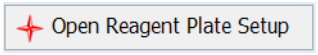
Note: If you have already manually prepared the reagents, you can skip to "Step 6. Stack prepared wash and lid plates" on page 7.

To run the Reagent Plate Setup:

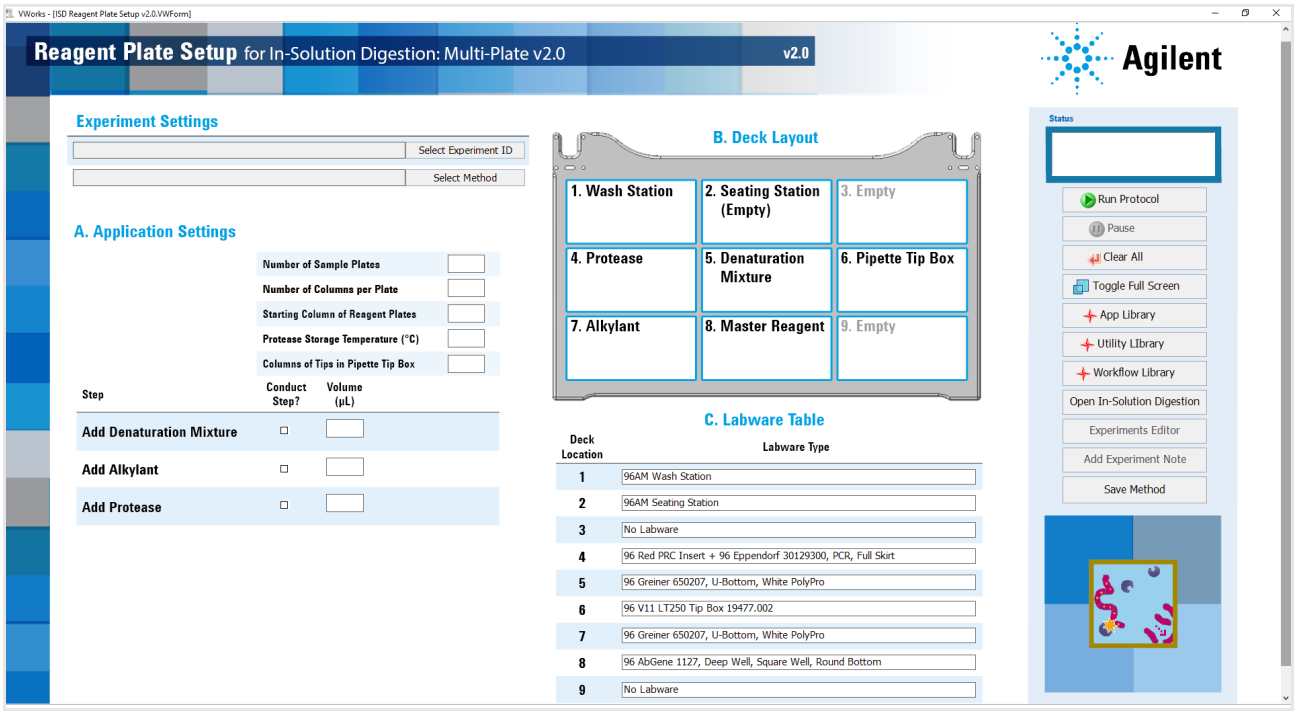
1. Open the **In-Solution Digestion: Multi-Plate v2.0** app.

   Note: For detailed instructions, see the user guide for this app.

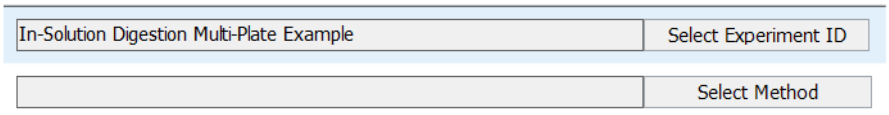
2. In the navigation pane on the right side of the form, click

   . The Reagent Plate Setup form 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 7.

   Note: Agilent provides a default method as a starting point for your method development. The inputs for this application vary widely depending on the intended use. See the *In-Solution Digestion: Multi-Plate User Guide* for guidance on developing a method that suits your needs.

6. To create or modify a method for Reagent Plate Setup:

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

a In the **Application Settings** area, select the settings for your run. For help, see, "Application Settings for Reagent Plate Setup" on page 6.

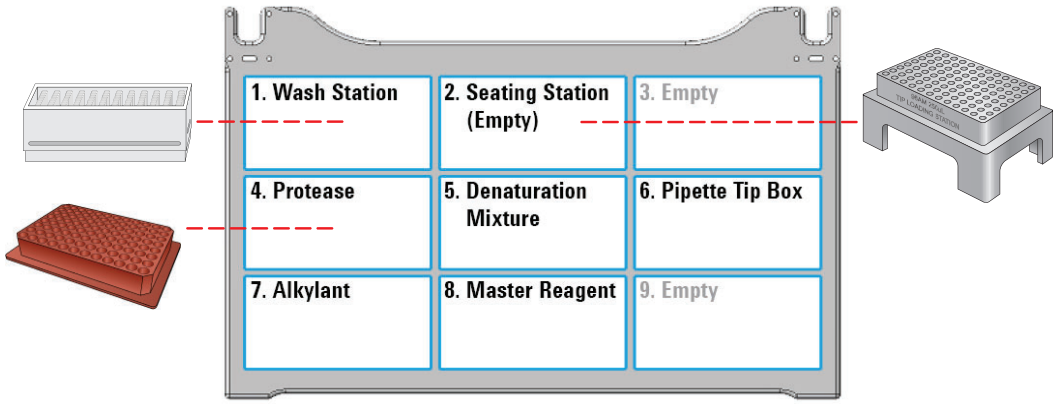
b To save the method, click Save Method. In the **Save File As** dialog box, type the file name and click **Save**.

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

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.**

7 Ensure that the reagent plates, pipette tips, and accessories are at the assigned deck locations, as shown in the **Deck Layout** image of the form.

At deck location **4**, ensure that the Red PCR Plate Insert is installed with the **Protease** plate. Otherwise, the protease will not be transferred properly.



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.**

8 Click Run Protocol to start the run.

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

Table **Application Settings for Reagent Plate Setup**

Setting or step	Description	Value	
Number of Sample Plates	Specifies the number of sample plates to be processed.	Default:	1
		Range:	1–4
Number of Columns per Plate	Specifies the number of columns in each reagent plate (Protease, Denaturation, and Alkylant) that will be filled with reagent aliquots from the Master Reagent plate. This number must be consistent with the number of columns of samples in each sample plate. The value is used with the Starting Column of Reagent Plates to determine which columns in the reagent plates will receive reagents.	Default:	1
		Range:	1–12
Starting Column of Reagent Plates	Defines the first column in each of the reagent plates that will receive reagent aliquots. This value works with the Number of Columns per Plate to define the range of each reagent plate that will receive reagent.	Default:	1
		Range:	1–12
Protease Storage Temperature	Specifies the temperature set-point that will be used for the Protease plate for the entire Reagent Plate Setup protocol. The temperature controller will not turn off after completion of the Reagent Plate Setup protocol. The assumption is that an In-Solution Digestion run will closely follow the Reagent Setup Run.	Default:	10 °C
		Range:	4–35 °C
Columns of Tips in Pipette Tip Box	Specifies the number of full columns of 250 µL pipette tips in the source tip box. The columns of pipette tips must be contiguous and contain 8 pipette tips per column. If specifying fewer than 12 columns, ensure that no pipette tips are present in the unspecified columns. Make sure that the empty columns are on the right side of the tip box.	Default:	12
		Range:	1–12
Add Denaturation Mixture	Transfers the specified volume, plus the required overage, to the Denaturation Mixture plate.	Default:	30 µL*
		Range:	1–250 µL
Add Alkylant	Transfers the specified volume, plus the required overage, to the Alkylant plate.	Default:	6 µL*
		Range:	1–250 µL
Add Protease	Transfers the specified volume, plus the required overage, to the Protease plate.	Default:	9 µL*
		Range:	1–250 µL

* Input the value for the volume that you want to add to the samples during the digestion run. The Reagent Volume Calculator automatically adjusts the value to include the recommended overage and provides the volume that you need to add to the plate to transfer the desired volume.

For example, if you set the Add Denaturation Mixture volume to 30 µL, then 50 µL will be added to each active well: 30 µL for the samples and 20 µL for overage.

Step 5. Clean up after Reagent Plate Setup

To clean up after the Reagent Plate Setup run:

1. Remove the seating station with the used tips (deck location 2), the Master Reagent plate (deck location 8), and the pipette tip box (deck location 6) from the Bravo deck.
2. Discard the excess Master Reagents and used pipette tips following appropriate waste disposal procedures.

IMPORTANT Do not remove the Protease plate from deck location 4, or the stack of reagent plates from deck location 7. These reagents are in their appropriate positions for the Digestion run.

Step 6. Stack prepared wash and lid plates

The automated Bravo plate stacking is required to create a perfectly aligned stack of wash and lid plates.

To stack the prepared wash and lid plates:

1. Open the **In-Solution Digestion: Multi-Plate** form.

   If the **Reagent Plate Setup for In-Solution Digestion: Multi-Plate** form is open, click Open In-Solution Digestion in the navigation pane.

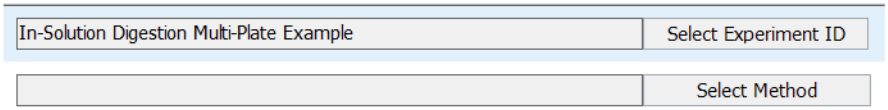
2. Click Run Plate Stacking in the navigation pane.

   Follow the instructions that appear on the screen after the run starts.

Step 7. Run Digestion protocol

To run the Digestion protocol:

1. If applicable, click **Select Experiment ID** to open the Experiments Editor.
2. In the **Experiments Editor**, select the **Experiment ID** that you want to use to capture the steps in this application, and then click **Use Selected**.



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

   To modify the selected method, proceed to step 4. Otherwise, go to step 5.

4. To create or modify a method for the In-Solution Digestion: Multi-Plate protocol:

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

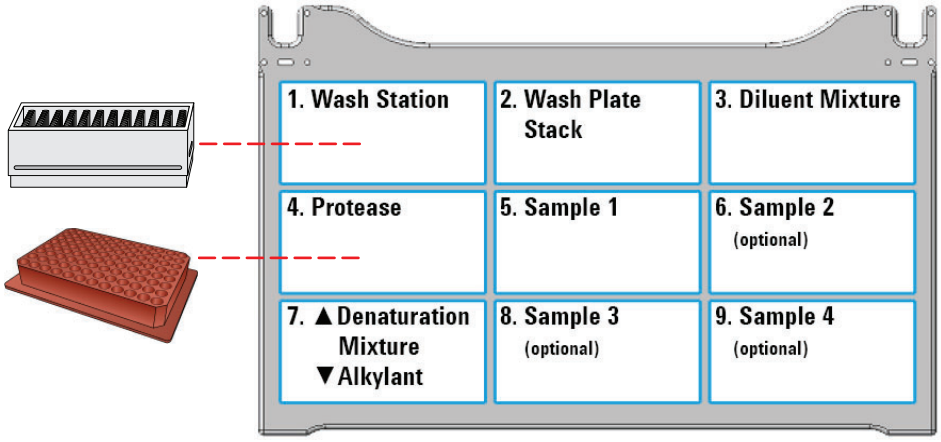
   a. In the **Application Settings** area, specify the sample information, protease storage temperature, and step settings.

      For help, see "Application Settings" on page 10.

   b. 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.

5 Ensure that the accessories and filled reagent plates are at the assigned deck locations, as shown in the **Deck Layout** image on the form.



At deck location **7**, ensure the **Denaturation Mixture** plate is stacked atop the **Alkylant** plate.

Note: The Reagent Plate Setup protocol stacks the labware at deck location 7 automatically. If you prepared the reagent plates manually, you must stack the plates manually at deck location 7.

IMPORTANT

Do not remove any of the reagent plates from the deck even if the reagent is not being used. Instead, you may use an empty microplate as a place holder. The protocol requires these labware to be in their defined positions to run properly.

The protocol requires five plates for the stack at deck location 2 regardless of the number of sample plates.

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.

6 Click 

to start the run.

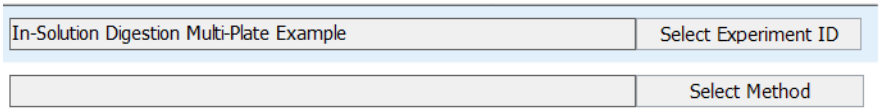
To monitor the progress of the run, check the **Status** box in the upper right corner of the form.

Step 8. 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**.



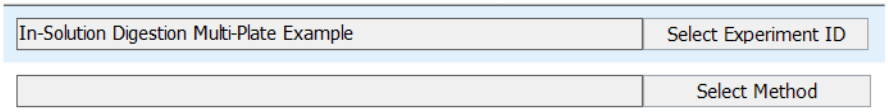
   d. Click **Select Method** to select and load the method for this utility.
   e. Confirm that the labware and accessories on the AssayMAP Bravo deck match the display in the **Deck Layout** area of the form.
   f. Click  to start the run.

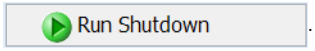
Step 9. Shut down at end of day

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  .

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.

Application Settings

The following table provides an overview of the settings and steps for the In-Solution Digestion: Multi-Plate app.

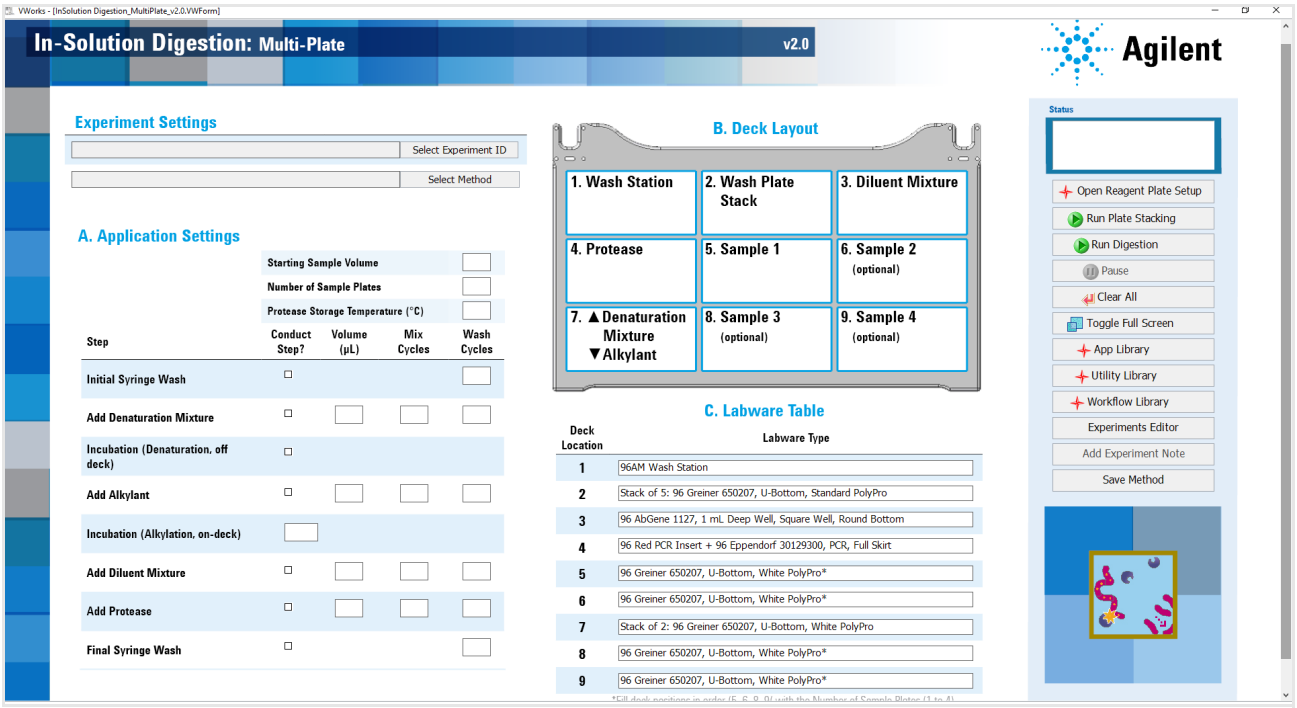


Table **In-Solution Digestion: Multi-Plate Application Settings overview**

Setting or Step*	Description	Value	
Starting Sample Volume	Specifies the volume of sample in each well in the sample plate at the beginning of the run.	Default:	15 µL
		Range:	0–300 µL
Number of Sample Plates	Specifies the number of sample plates on the AssayMAP Bravo deck.	Default:	1
		Range:	1–4
Protease Storage Temperature	Specifies the temperature set-point for the Protease plate (deck location 4) for the duration of the run. The temperature controller will turn off after completion of the In-Solution Digestion run. *Note:* The temperature of the wells will be slightly different than the Peltier set point.	Default:	10 °C
		Range:	4–37 °C

Table **In-Solution Digestion: Multi-Plate Application Settings overview (continued)**

Setting or Step*	Description		Volume in µL	Mix Cycles	Wash Cycles
Initial Syringe Wash	Washes syringes at the wash station.	Default:	–	–	3
		Range:	–	–	1–10
Add Denaturation Mixture	Aspirates the Denaturation Mixture (deck location 7) into the syringes, and then dispenses it into Sample plate 1 (deck location 5). The solutions in Sample plate 1 are mixed based on the Mix Cycles value. The the corresponding Syringe Wash plate is used to perform a Stringent Syringe Wash. This step repeats for each sample plate that is on the deck (locations 6, 8, and 9).	Default:	30	15	3
		Range:	1–250	0–30	0–10
Incubation (Denaturation, off-deck)	Pauses the run after the Add Denaturation Mixture step so that you can manually move the sample plates off deck for incubation, if required, for denaturation or reduction. After incubation, you manually place the sample plates back onto the Bravo deck and resume the protocol run.	Default:	Selected		
		Range:	Selected, Not selected		
Add Alkylant	Moves the Denaturation Mixture plate from deck location 7 to 3, aspirates the Alkylant (deck location 7) into the syringes, and then dispenses it into Sample plate 1 (deck location 5). The solutions in Sample plate 1 are mixed based on the Mix Cycles value. The corresponding Syringe Wash plate is used to perform a Stringent Syringe Wash. This step repeats for each sample plate that is on the deck (locations 6, 8, and 9).	Default:	6	15	3
		Range:	1–250	0–30	0–10
Incubation (Alkylation, on-deck)	Incubates the sample plates for the specified period (in minutes) on the deck at room temperature.	Default:	Time: 45 minutes		
		Range:	0–180 minutes		
Add Diluent Mixture	Moves the Denaturation Mixture plate from deck location 3 to 7, aspirates the Diluent Mixture (deck location 3) into the syringes, and then dispenses it into Sample plate 1 (deck location 5). The solutions in Sample plate 1 are mixed based on the Mix Cycles value. The corresponding Syringe Wash plate is used to perform a Stringent Syringe Wash. This step repeats for each sample plate that is on the deck (locations 6, 8, and 9).	Default:	210	15	3
		Range:	1–250	0–30	0–10

Setting or Step*	Description		Volume in µL	Mix Cycles	Wash Cycles
Add Protease	Aspirates Protease (deck location 4) into the syringes, and then dispenses it into Sample plate 1 (deck location 5). The solutions in Sample plate 1 are mixed based on the Mix Cycles value. The corresponding Syringe Wash plate is used to perform a Stringent Syringe Wash. This step repeats for each sample plate that is on the deck (locations 6, 8, and 9).	Default:	9	15	3
		Range:	1–250	0–30	0–10
Final Syringe Wash	Washes the syringes at the wash station (deck location 1).	Default:	–	–	3
		Range:	–	–	1–10

*Additional guidelines can be found in the user guide for this application. Go to the *Assay development guidelines and protocol notes* topic and see the *Protocol stepwise guidelines* section.

A complete list of the robotic movements executed during a run can also be found in the user guide in the *Assay development guidelines and protocol notes* topic. See the *Automation movements during the protocol* section for details.

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D0010507, Revision A
May 2022