AssayMAP Protein Sample Prep Workbench

In-Solution Digestion: Multi-Plate v1.1 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 protocols 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 **Reagent Volume Calculator** to determine the reagent composition and volume requirements by filling out the **Reagent Setup** worksheet.

The calculator automatically displays the:

- Total volume of the reagents required and the volume of the components required to generate these reagents.
- Plate setup and 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

Prepare the reagents according to the recipes shown in the **Reagent Prep** worksheet of the **Reagent Volume Calculator**.

CAUTION

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

The Reagent Volume Calculator automatically includes the required excess volume per well. You can also find 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. Set up the reagent and sample plates

You can set up the reagent plates for the In-Solution Digestion: Multiplate app automatically using the Reagent Plate Setup, or manually using hand pipettes:

- *Automated Reagent Plate Setup*. 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 will be 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.

Note: If you manually prepare your reagent plates at this step, you will skip Step 6. Run the Reagent Plate Setup protocol and Step 7. Clean up after the reagent setup.

Step 4. 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 5. Prepare plates for stacking

Before running the In-Solution Digestion protocol, you must stack five 96-well Greiner 650207 U-Bottom plates at AssayMAP Bravo deck location 2. The plate stacking is a requirement even if you do not run the Add Protease step 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 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.

Step 6. Run the Reagent Plate Setup protocol

Note: If you have already manually prepared the reagents, you can skip to "Step 8. Stacking the wash and lid plates" on page 5.

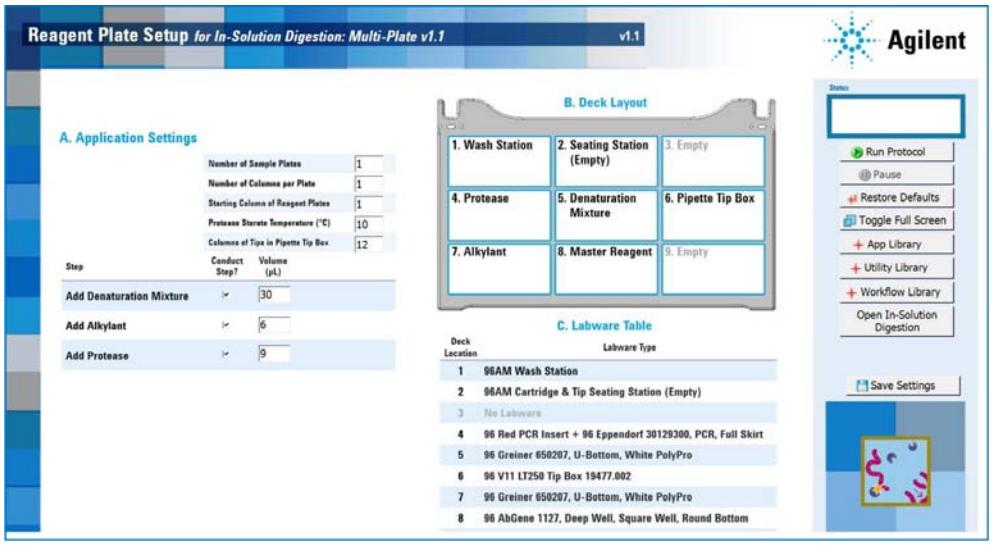
To run the Reagent Plate Setup:

1. Open the **In-Solution Digestion: Multi-Plate v1.1** app.

   Note: You can open In-Solution Digestion: Multiplate v1.1 from the App Library or the Workflow section of the Protein Sample Prep Workbench.

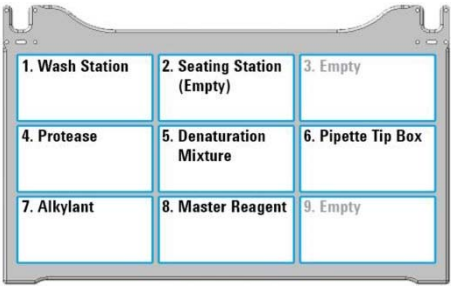
2. Click 

to display the Reagent Plate Setup form.



3 Select the **Application Settings** appropriate for your run. For details, see the table, "Application Settings for Reagent Plate Setup" on page 4.

4 Place the reagent plates and tips at the assigned deck locations, as the **Deck Layout** shows. Ensure that the labware are properly seated.



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 **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.**

IMPORTANT Ensure that the Red PCR Plate Insert is installed with the Protease plate at deck location 4. Otherwise, the protease will not be transferred properly.

5 Ensure the labware exactly matches the display in the **Labware Table**.

CAUTION **Incorrect labware can cause a hardware collision, resulting in equipment damage. Ensure that the physical labware present on the Bravo deck exactly matches the Labware Table.**

6 Click **Run Protocol**. The Reagent Plate Setup protocol run starts.

Table Application Settings for Reagent Plate Setup

Setting or step	Description	Default value (range)
Number of Sample Plates	Specifies the number of sample plates to be processed.	1 (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. This should be consistent with the number of columns that contain 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 contain reagents.	1 (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 should be filled with aliquots. The value in this box enables efficient use of labware.	1 (1–12)
Protease Storage Temperature (°C)	Specifies the temperature set-point that will be used for the Protease plate for the entire Reagent Plate Setup protocol. *Note:* 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.	10 °C (4–35 °C)
Columns of Tips in Source Tip Box	Specifies the number of full columns of tips in the source tip box. The columns of tips must be contiguous and contain 8 tips per column. If specifying fewer than 12 columns, ensure that no tips are present in the unspecified columns. Make sure that the empty columns are on the right side of the box.	12 (1–12)
Add Denaturation Mixture	Transfers the specified volume plus the required overage to the Denaturation Mixture plate. The software automatically calculates the volume (including overages) to be transfered from the Denature Master mixture to each well of the Denaturation Mixture plate. This step is selected by default. To skip this step, clear the check box.	30 µL* (1–250 µL)
Add Alkylant	Transfers the specified volume plus the required overage to the Alkylant plate. The software automatically calculates the volume (including overages) to be transfered from the Alkylant Master mixture to each well of the Alkylant plate. This step is selected by default. To skip this step, clear the check box.	6 µL* (1–250 µL)
Add Protease	Transfers the specified volume plus the required overage to the Protease plate. The software automatically calculates the volume (including overages) to be transfered from the Protease Master mixture to each well of the Protease plate. This step is selected by default. To skip this step, clear the check box.	9 µL* (1–250 µL)
* Use the values from the Reagent Volume Calculator. The total volume of Denaturation Mixture, Alkylant, Protease, Diluent Mixture, and sample must be less than 300 µL to accommodate the working volume of the labware. Be sure to include any liquid that will be added to the sample plate after digestion.		

Step 7. Clean up after the reagent setup

To clean up after the run:

1. Remove the 96AM Cartridge & Tip 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 In-Solution Digestion run.

Step 8. Stacking the wash and lid plates

To stack the prepared wash and lid plates:

1. On the **Reagent Plate Setup for In-Solution Digestion** page, click **Open In-Solution Digestion**.
2. On the **In-Solution Digestion** page, click **Run Plate Stacking**. Follow the instructions that appear on the screen after the run starts.

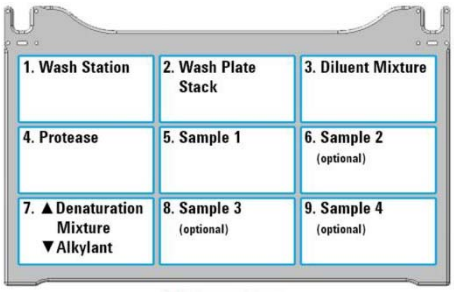
Step 9. Run the Digestion protocol

To run the Digestion protocol:

1. On the **In-Solution Digestion** page, select the **Application Settings** for your run. For details, see "In-Solution Digestion settings" on page 6.
2. Ensure that all labware are at the assigned deck locations, as the **Deck Layout** shows. Ensure the labware are properly seated.

   At deck location 7, ensure the Denaturation Mixture plate is stacked atop the Alkylation 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

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.

3. Ensure the labware exactly matches the display in the **Labware Table**.

CAUTION

Incorrect labware can cause a hardware collision, resulting in equipment damage. Ensure that the physical labware present on the Bravo deck exactly matches the Labware Table.

4. Click **Run Digestion** to start the run.

Step 10. Clean up after each run

To clean up after the run:

1. Remove used labware from the deck.
2. Discard the leftover reagents appropriately.
3. *Optional*. To conduct stringent washing of the syringes, run the **Syringe Wash** utility  .

Step 11. 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.

In-Solution Digestion settings

The following figure shows the application interface, and the following table provides the settings and step descriptions.

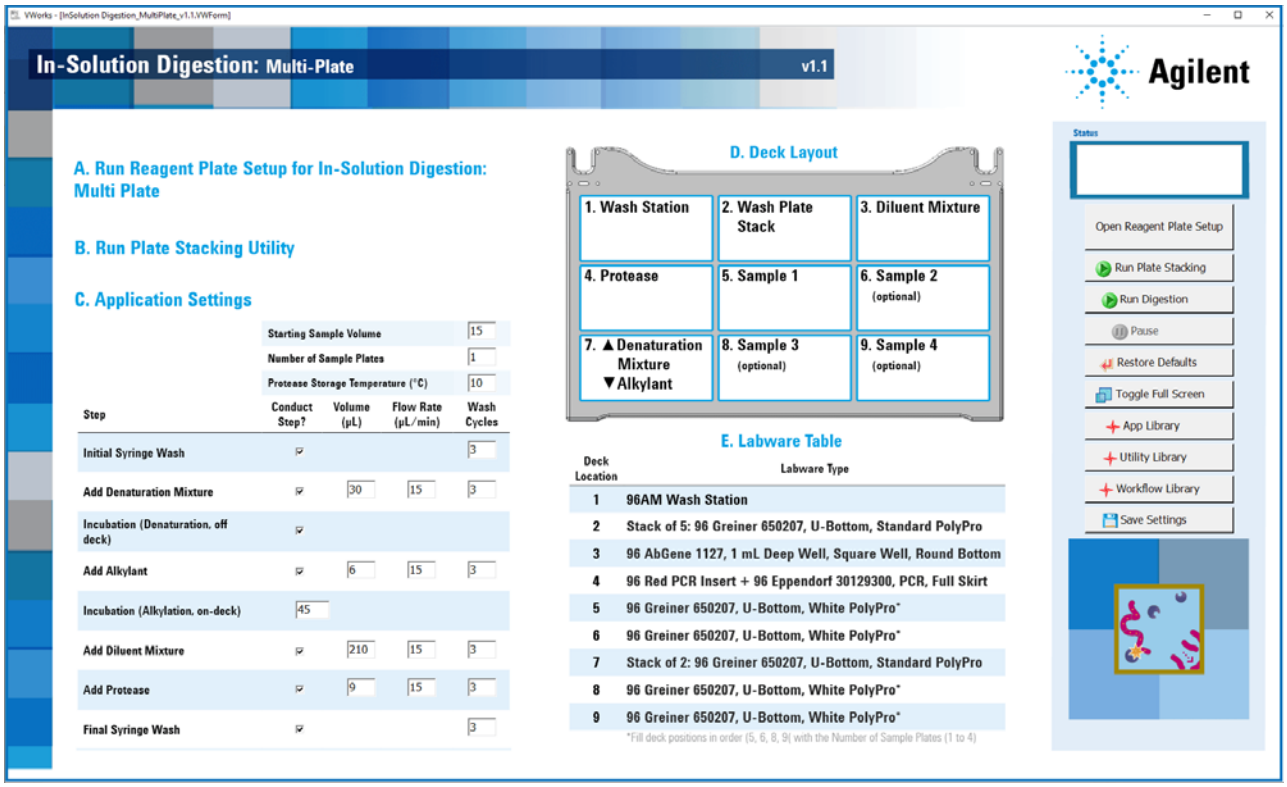


Table In-Solution Digestion Application Settings

Step	Description	Volume in µL	Mix Cycles	Wash Cycles
Starting Sample Volume (µL)	The volume of sample in each well in the sample plate.	15 µL (0–300 µL)		
Number of Sample Plates	The number of sample plates on the AssayMAP Bravo deck.	1 (1–4)		

Step	Description	Volume in µL	Mix Cycles	Wash Cycles
Protease Storage Temperature (°C)	The temperature set-point that will be used for the Protease plate for the entire In-Solution Digestion run. The temperature controller will turn off after completion of the In-Solution Digestion run.	10 °C (4–37 °C)		

Table In-Solution Digestion Step Settings

Step	Description	Volume in µL	Mix Cycles	Wash Cycles
Initial Syringe Wash	Washes syringes at the wash station (deck location 1).	–	–	3 (1–10)
Add Denaturation Mixture	Adds the Denaturation Mixture to the sample wells in each sample plate. The syringes are washed between sample plates.	30 (1–250)	15 (0–30)	3 (0–10)
Incubation (Denaturation, off-deck)	Pauses the run 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.			
Add Alkylant	Adds the Alkylant to each sample plate. The syringes are washed between sample plates.	6 (1–250)	15 (0–30)	3 (0–10)
Incubation (Alkylation, on-deck)	Incubates the lidded sample plates for the specified period (in minutes) on the deck. During incubation, alkylation occurs.	Time: 45 minutes (0–180 minutes)		
Add Diluent Mixture	Adds the Diluent Mixture to each sample plate. The syringes are washed between sample plates.	210 (1–250)	15 (0–30)	3 (0–10)
Add Protease	Adds the Protease to each sample plate for enzymatic digestion. The syringes are washed between sample plates.	9 (1–250)	15 (0–30)	3 (0–10)
Final Syringe Wash	Washes the syringes at the wash station (deck location 1).	–	–	3 (1–10)

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