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**

Open the Reagent Volume Calculator for the In-Solution Digestion: Multi-Plate application, and fill out the Reagent Setup worksheet to define the digestion run. The calculator automatically displays the required reagent preparation volumes in the Reagent Prep, Automated Plate Setup, and Manual Plate Setup worksheets. 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 volume excess is required in all labware types to ensure proper volume transfer. Use the Reagent Volume Calculator to automatically include excess volume, or to look up recommended values for each allowable labware type.

**Step 3. Set up the reagent and sample plates**

You can set up the reagent plates automatically using the Reagent Plate Setup for In-Solution Digestion App, 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 steps 6 and 7.

**Step 4. Start up the system**

**To start up the system:**

1. Check the levels of the wash station source and waste carboys, and fill or empty as required.

2. Turn on the AssayMAP Bravo Platform, Pump Module, and the Peltier Thermal Station Controller, if included.

3. Start the Protein Sample Prep Workbench, and open the Utility Library.
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 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 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 4.

To run the Reagent Plate Setup:
1. Open the In-Solution Digestion: Multi-Plate v1.0 application. When the application opens, the Reagent Plate Setup page appears.

Note: You can open the application from the App Library or the Workflow section of the Protein Sample Prep Workbench.
2. Select the Application Settings appropriate for your run. For details, see the table, “Application Settings for Reagent Plate Setup” on page 3.
Step 6. Run the Reagent Plate Setup protocol

3 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.

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.

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.

4 Ensure the labware exactly matches the display in the Labware Table.

5 Click Run Reagent Setup. The protocol run starts.

Table Application Settings for Reagent Plate Setup

<table>
<thead>
<tr>
<th>Setting or step</th>
<th>Description</th>
<th>Default value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Sample Plates</td>
<td>Specifies the number of sample plates to be processed.</td>
<td>1 (1–4)</td>
</tr>
<tr>
<td>Number of Columns per Plate</td>
<td>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.</td>
<td>1 (1–12)</td>
</tr>
<tr>
<td>Starting Column of Reagent Plates</td>
<td>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.</td>
<td>1 (1–12)</td>
</tr>
<tr>
<td>Protease Storage Temperature (°C)</td>
<td>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, assuming that an In-Solution Digestion run will closely follow.</td>
<td>10 °C (4–35 °C)</td>
</tr>
</tbody>
</table>
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, the Master Reagent plate, and the unused pipette tips 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 5.
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.

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### 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.

<table>
<thead>
<tr>
<th>Setting or step</th>
<th>Description</th>
<th>Default value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns of Tips in Source Tip Box</td>
<td>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.</td>
<td>12 (1–12)</td>
</tr>
<tr>
<td>Add Denaturation Mixture</td>
<td>Transfers the specified volume to the Denaturation Mixture plate. The software automatically calculates the volume (including overages) to be transferred 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.</td>
<td>30 µL* (1–250 µL)</td>
</tr>
<tr>
<td>Add Alkylant</td>
<td>Transfers the specified volume to the Alkylation plate. The software automatically calculates the volume (including overages) to be transferred from the Alkylation Master mixture to each well of the Alkylation plate. This step is selected by default. To skip this step, clear the check box.</td>
<td>6 µL* (1–250 µL)</td>
</tr>
<tr>
<td>Add Protease</td>
<td>Transfers the specified volume to the Protease plate. The software automatically calculates the volume (including overages) to be transferred 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.</td>
<td>9 µL* (1–250 µL)</td>
</tr>
</tbody>
</table>

* 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 10. Clean up after each run

To clean up after the run:
1. Remove used labware from the deck, and clean up any spills.
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 the 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>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
<th>Default value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting Sample Volume (µL)</td>
<td>The volume of sample in each well in the sample plate.</td>
<td>15 µL (0–300 µL)</td>
</tr>
<tr>
<td>Number of Sample Plates</td>
<td>The number of sample plates on the AssayMAP Bravo deck.</td>
<td>1 (1–4)</td>
</tr>
<tr>
<td>Protease Storage Temperature (°C)</td>
<td>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.</td>
<td>10 °C (4–37 °C)</td>
</tr>
<tr>
<td>Step</td>
<td>Description</td>
<td>Volume in µL</td>
</tr>
<tr>
<td>Initial Syringe Wash</td>
<td>Washes syringes at the wash station (deck location 1).</td>
<td>–</td>
</tr>
<tr>
<td>Add Denaturation Mixture</td>
<td>Adds the Denaturation Mixture to each sample plate. The syringes are washed between sample plates.</td>
<td>30 (1–250)</td>
</tr>
<tr>
<td>Incubation (Denaturation, off-deck)</td>
<td>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.</td>
<td>–</td>
</tr>
<tr>
<td>Add Alkylant</td>
<td>Adds the Alkylant to each sample plate. The syringes are washed between sample plates.</td>
<td>6 (1–250)</td>
</tr>
<tr>
<td>Incubation (Alkylation, on-deck)</td>
<td>Incubates the lidded sample plates for the specified period (in minutes) on the deck. During incubation, alkylation occurs.</td>
<td>Time: 45 minutes (0–180 minutes)</td>
</tr>
<tr>
<td>Add Diluent Mixture</td>
<td>Adds the Diluent Mixture to each sample plate. The syringes are washed between sample plates.</td>
<td>210 (1–250)</td>
</tr>
<tr>
<td>Add Protease</td>
<td>Adds the Protease to each sample plate for enzymatic digestion. The syringes are washed between sample plates.</td>
<td>9 (1–250)</td>
</tr>
<tr>
<td>Final Syringe Wash</td>
<td>Washes the syringes at the wash station (deck location 1).</td>
<td>–</td>
</tr>
</tbody>
</table>