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 your run**

Use the **Reagent Volume Calculator** for In-Solution Digestion: Single Plate v1.1 to:
- Determine reagent volume preparation requirements
- Make labware selections

For in-depth assay development guidelines, see the **In-Solution Digestion: Single Plate v1.1 User Guide** in the Literature Library of the Protein Sample Prep Workbench.

**Step 2. Prepare sample and reagent plates**

Fill the sample and reagent plates immediately before run time to minimize evaporation.

**CAUTION**

A small sample and 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 recommended values for each allowable labware type in the user guide.

**Step 3. 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**.
4. Open the **System Startup/Shutdown utility**.
5. Click **Run Startup** to initialize the AssayMAP Bravo Platform and accessories.

**WARNING**

When you initialize the Bravo Platform, the head and tie bar can move. To prevent injury, keep clear of the device while it is in motion.

6. During the Startup protocol, verify that all the wash station chimneys have liquid flowing through them.
Step 4. Prepare the tip seating station

*Runs using pipette tips only.* If pipette tips are required for the run, fill the 96AM Cartridge & Tip Seating Station with the appropriate number and configuration of pipette tips to match the samples to be processed. To prepare the pipette tips, run the Pipette Tip Transfer utility.

Step 5. Run the application

*To run the In-Solution Digestion: Single Plate application:*

1. Open the *In-Solution Digestion: Single Plate v1.1* App.
2. To create a new method, skip to step 3.

To use or modify an existing method, click  in the A: Select Method area, select the method, and then click Load Values to display all the settings and the Deck Layout associated with the selected method.

To run a saved method exactly as written, skip to step 7. Otherwise, proceed to step 3 to make changes.

3. Under B. Input Sample Settings, verify the settings, or make any required changes to meet the needs of your run. For more details, see “Application overview” on page 3.

4. Under C. Input Addition Step Settings, verify the settings, or make any required changes to meet the needs of your run. For more details, see “Application overview” on page 3.

5. Under D. Update Deck Layout, click Update Deck Layout to refresh the deck so that it reflects any changes that you made to the method.

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

6. To save a new or edited method, specify the new Name or select Overwrite if Name Exists in the Save Method area, and then click Save.

7. Place the items at the assigned deck locations, as shown in the Deck Layout of the App interface:
   - Place the filled sample plate at deck location 4, and place the filled reagent plates at their respective deck locations.
   - If lids are specified in the method, ensure that the lids are on the corresponding plates.
   - If pipette tips are required for the run, place the prepared 96AM Cartridge & Tip Seating Station at deck location 2.

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.

8. Ensure that all labware are properly seated on the deck.

Impropersly 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.

9. Click Run Digestion to start the run.
Step 6. 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 7. 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.

Application overview

The application includes up to four generic liquid-handling steps that successively transfer a reagent from a reagent plate at deck location 5, 6, 8, or 9, into the Sample plate at deck location 4. The following figure shows the interface, and the following tables provide the settings and step descriptions.

Table Sample settings and step descriptions

<table>
<thead>
<tr>
<th>Sample setting</th>
<th>Description</th>
<th>Default value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Plate Labware</td>
<td>Specifies the type of labware that will be placed at deck location 4, which contains the sample to be digested.</td>
<td>–</td>
</tr>
</tbody>
</table>
### Table: Addition step settings and descriptions

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
<th>Default value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Plate Lidded</td>
<td>Specifies whether the Sample Plate has a lid. If selected, the Sample plate must have a lid present at the start of the run. Clear the check box if the Sample Plate will not have a lid.</td>
<td>–</td>
</tr>
<tr>
<td>Starting Sample Volume (µL)</td>
<td>Specifies the volume of sample that is initially present in the Sample Plate at the beginning of the run.</td>
<td>0 µL (0–1000 µL)</td>
</tr>
<tr>
<td>Number of Full Columns of Samples</td>
<td>Specifies the number of full columns of samples in the digestion plate.</td>
<td>1 (1–12)</td>
</tr>
<tr>
<td>Addition Number</td>
<td>Indicates the order in which the addition steps will be executed.</td>
<td>These values are fixed.</td>
</tr>
<tr>
<td>Addition Name</td>
<td>Specifies a name used to uniquely identify the addition step. Select a name from the list or enter a new name.</td>
<td>–</td>
</tr>
<tr>
<td>Reagent Deck Location</td>
<td>Indicates the deck location from which liquid will be drawn when this step runs.</td>
<td>This value is fixed.</td>
</tr>
<tr>
<td>Addition Volume (µL)</td>
<td>Specifies the volume of each reagent that will be transferred from its respective reagent plate, into the Sample plate.</td>
<td>10 µL (0–1000 µL)</td>
</tr>
<tr>
<td>Mixing Cycles</td>
<td>Specifies the number of mixing cycles that will be used to homogenize the combined samples and reagents after reagent addition.</td>
<td>3 (0–10)</td>
</tr>
<tr>
<td>Incubation Time (min)</td>
<td>Specifies the amount of time that the Sample plate will be incubated (deck location 4) after receiving a reagent aliquot.</td>
<td>0 (0–120 min)</td>
</tr>
<tr>
<td>Incubation Temp (°C)</td>
<td>Specifies the temperature set point of the Peltier Thermal Station during the Sample plate incubation (deck location 4).</td>
<td>OFF (OFF, 4–70 °C)</td>
</tr>
<tr>
<td>Pause After Addition</td>
<td>Pauses the protocol after completing the wash cycles and before starting the next addition step. See the In-Solution Digestion: Single Plate v1.1 User Guide for instructions on effectively using the Pause feature.</td>
<td>–</td>
</tr>
<tr>
<td>Labware Selection</td>
<td>Specifies the labware type that will be used for each addition step.</td>
<td>–</td>
</tr>
<tr>
<td>Plate Lidded</td>
<td>Specifies whether the labware has a lid. If selected, the corresponding plate must have a lid present at the start of the run. Clear the check box if the plate will not have a lid.</td>
<td>–</td>
</tr>
<tr>
<td>Use Tips for Addition</td>
<td>Specifies whether pipette tips (deck location 2) will be used instead of the bare probes to add this reagent into the Sample plate. To use tips, select the check box. To use the bare probes, clear the check box.</td>
<td>–</td>
</tr>
<tr>
<td>Number of Wash Cycles</td>
<td>Specifies the number of wash cycles after the reagent addition step has completed. If the Use Tips for Addition check box is selected, this step washes the pipette tips. If the Use Tips for Addition check box is cleared, the protocol washes the bare syringe probes.</td>
<td>3 (1 - 10)</td>
</tr>
</tbody>
</table>