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 Affinity Purification v1.0 Reagent Volume Calculator to:
- Determine reagent volume preparation requirements
- Make labware selections

For in-depth assay development guidelines, see the Affinity Purification v1.0 Users Guide in the Literature Library of the Protein Sample Prep Workbench.

**Step 2. Prepare reagents plates**

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

**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 to look up recommended values for each allowable labware type.

**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. Run the application**

To run the application:

1. Run the Cartridge Transfer v1.0 utility to set up the cartridges
Step 5. Clean up after each run

2. Open the **Affinity Purification v1.0** App.

3. Under **Application Settings**, select the settings appropriate for your run. For help, see “Application Settings” on page 3.

**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.

4. Place filled reagent plates at the assigned deck locations, as shown in the **Deck Layout** of the App interface.

**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.

5. In the **Labware Table** of the App interface, select the labware that is required for your run.

**CAUTION**

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

6. Click **Run Affinity Purification** to start the run.

**To clean up after the run:**

1. Remove used labware from the deck, and clean up any spills.

2. Discard leftover reagents appropriately.

3. **Optional.** To conduct stringent washing of the syringes, run the **Syringe Wash** utility.

**Step 6. 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 Settings

The following tables provide an overview of the Application Settings section in the Affinity Purification v1.0 App.

## Application Settings overview

<table>
<thead>
<tr>
<th>Setting</th>
<th>Description</th>
<th>Default value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Full Columns of Cartridges</td>
<td>Specifies the number of full columns in the cartridge holder at deck location 2.</td>
<td>1 (1–12)</td>
</tr>
</tbody>
</table>

## Steps

<table>
<thead>
<tr>
<th>Steps</th>
<th>Description</th>
<th>Volume (µL)</th>
<th>Flow Rate (µL/min)</th>
<th>Wash Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Syringe Wash</td>
<td>Washes syringes at the wash station (deck location 1).</td>
<td>–</td>
<td>–</td>
<td>3 (0–10)</td>
</tr>
<tr>
<td>Prime</td>
<td>Aspirates Priming Buffer (deck location 3) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1).</td>
<td>100 (0–250)</td>
<td>300 (0.5–500)</td>
<td>1 (0–10)</td>
</tr>
<tr>
<td>Equilibrate</td>
<td>Aspirates Equilibration Buffer (deck location 3) into the syringes, and then dispenses it through the cartridges into the wash station (deck location 1).</td>
<td>50 (0–250)</td>
<td>10 (0.5–500)</td>
<td>1 (0–10)</td>
</tr>
<tr>
<td>Load Sample</td>
<td>Aspirates samples (deck location 4) into the syringes, and then dispenses them through the cartridges into the Flow Through Collection (deck location 7) or into the wash station (deck location 1).</td>
<td>100 (0–1000)</td>
<td>5 (0.1–500)</td>
<td>3 (0–10)</td>
</tr>
<tr>
<td>Collect Flow Through</td>
<td>If selected, collects the sample flow-through at the Flow Through Collection (deck location 7). If not selected, discards the sample flow-through at the wash station (deck location 1).</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steps</td>
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<td>Volume (µL)</td>
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</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------</td>
<td>--------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Cup Wash 1</td>
<td>Rinses the cartridge cups with Cartridge Wash Buffer 1 (deck location 5), and then discards the liquid into the wash station (deck location 1).</td>
<td>25 (0–100)</td>
<td>–</td>
<td>1 (0–10)</td>
</tr>
<tr>
<td>Internal Cartridge Wash 1</td>
<td>Aspirates Cartridge Wash Buffer 1 (deck location 5) into the syringes, and then dispenses it through the cartridges into the Flow Through Collection (deck location 7) or into the wash station (deck location 1).</td>
<td>50 (0–250)</td>
<td>10 (0.5–500)</td>
<td>3 (0–10)</td>
</tr>
<tr>
<td>Collect Flow Through</td>
<td>If selected, collects the Internal Cartridge Wash 1 flow-through at the Flow Through Collection (deck location 7). If not selected, discards the Internal Cartridge Wash flow-through at the wash station (deck location 1).</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cup Wash 2</td>
<td>Rinses the cartridge cups with Cartridge Wash Buffer 2 (deck location 6) and discards the liquid into the wash station (deck location 1).</td>
<td>25 (0–100)</td>
<td>–</td>
<td>1 (0–10)</td>
</tr>
<tr>
<td>Internal Cartridge Wash 2</td>
<td>Aspirates Cartridge Wash Buffer 2 (deck location 6) into the syringes, and then dispenses it through the cartridges into the Flow Through Collection (deck location 7) or into the wash station (deck location 1).</td>
<td>50 (0–250)</td>
<td>10 (0.5–500)</td>
<td>3 (0–10)</td>
</tr>
<tr>
<td>Collect Flow Through</td>
<td>If selected, collects the Internal Cartridge Wash 2 flow-through at the Flow Through Collection (deck location 7). If not selected, discards the Internal Cartridge Wash 2 flow-through at the wash station (deck location 1).</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stringent Syringe Wash</td>
<td>Aspirates Syringe Wash Buffer (deck location 8) into the syringes, and then discards the liquid into the wash station (deck location 1).</td>
<td>50 (0–250)</td>
<td>–</td>
<td>1 (0–10)</td>
</tr>
<tr>
<td>Elute</td>
<td>Aspirates Elution Buffer (deck location 8) into the syringes, and then dispenses it through the cartridges into the Eluate Collection (deck location 9).</td>
<td>25 (0–250)</td>
<td>5 (0.5–500)</td>
<td>1 (0–10)</td>
</tr>
<tr>
<td>Eluate Discard</td>
<td>If selected, a specified initial volume of Eluate will be dispensed through the cartridges, and collected at the Flow Through Collection (deck location 7), or discarded at the wash station (deck location 1).</td>
<td>0 (0–25)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Add to Flow Through</td>
<td>If selected, collects the Eluate Discard in the Flow Through Collection (deck location 7). If not selected, discards the Eluate Discard at the wash station (deck location 1).</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Existing Collection Volume</td>
<td>Specifies the volume of liquid initially present in the Eluate Collection plate (deck location 9).</td>
<td>0 (0–300)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Final Syringe Wash</td>
<td>Washes the syringes at the wash station (deck location 1).</td>
<td>–</td>
<td>–</td>
<td>3 (0–10)</td>
</tr>
</tbody>
</table>