Protocol

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Affinity Purification Protocol

Turn On

Turn on the Agilent AssayMAP Bravo Platform, Pump Module 2.0 and Inheco Single TEC Controller.

Check

- Ensure the de-ionized (DI) water bottle is full.
- Ensure the waste bottle is empty.

Startup Utility

The first thing to do when you approach the AssayMAP Bravo in an unknown state, is to run the **Startup Utility**.

1. Open the **Protein Sample Prep Workbench** software using the desktop icon and select **Utility Library**.
2. Open the **Startup/Shutdown** utility by clicking on **Utility**.
3. Click **Run Startup** to run the startup utility with the default settings and read the notifications.

Affinity Purification

For Affinity Purification, we will do the following tasks with AssayMAP applications:

1. Transfer PA-W cartridge using **Cartridge Transfer**.
2. Purify mAb from CHO medium using **Affinity Purification**.
3. Neutralize sample using **Reagent Transfer**.

Transfer PA-W Cartridge

1. Navigate to **Workflow Library**, and open **Affinity Purification Workflow**.
2. Under **Utilities**, click **Cartridge Transfer** and transfer one column of PA-W cartridge to the 1st column of the cartridge seating station (Figure 1).

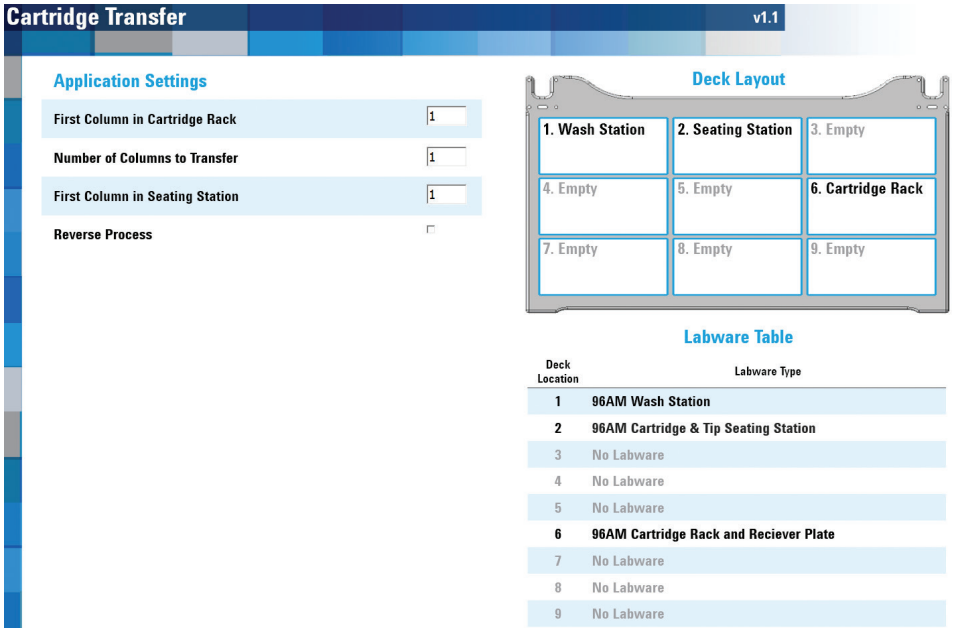

Figure 1. Cartridge Transfer parameters.

3. Depending on how many samples you have, the AssayMAP Bravo works on samples column by column, with each column containing up to eight samples. We take one full column as an example here.

Purify mAb from CHO Medium

4. Navigate back to Affinity Purification Workflow and open Affinity Purification.
5. Set parameters as shown in Figure 2.

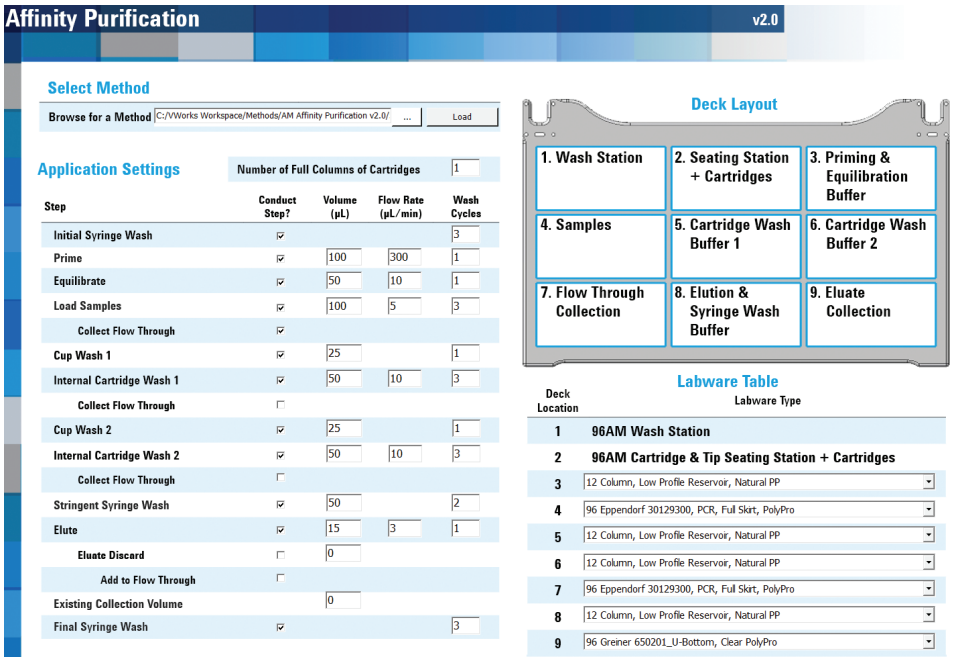

Figure 2. Affinity Purification parameters.

6. Transfer the following buffer and sample into its corresponding labware and place the labware in the deck location as indicated in the software interface.
   - **Sample** (deck location 4): Label a 96-well Eppendorf plate as “Sample Source Plate”. Transfer 110 µL of sample (0.5 µg/µL) into the plate according to the following layout. Cover the plate with a lid. (Lid will be removed before running the protocol).

	1	2
A	Sample 1	
B	Sample 1	
C	Sample 1	
D	Sample 2	
E	Sample 2	
F	Sample 2	
G	CHO Medium	
H	CHO Medium	

   - For **Prime & Equilibrate Buffer** (deck location 3): Label a 12-well reservoir as “PBS”. Manually fill the 1st column of the plate with **4.5 mL 1xPBS, pH=7.4**.
   - For **Cartridge Wash Buffer 1** (deck location 5): Label a 12-well reservoir as “1 M NaCl”. Manually fill the 1st column of the plate with **4 mL 1 M NaCl in 1XPBS, pH=7.4**.

- For **Cartridge Wash Buffer 2** (deck location 6): Label a 12-well reservoir as “PBS”. Manually fill the 1st column of the plate with **4 mL 1xPBS, pH=7.4**.
- For **Flow Through Collection** (deck location 7): Label a 96-well Eppendorf plate as “Flow through”. Place the labeled plate on deck location 7.
- For **Elution & Syringe Wash Buffer** (deck location 8): Label a 12-well reservoir as “5 % Acetic Acid”. Manually fill the 1st column of a 12-well reservoir with **4 mL 5 % acetic acid**.

  Note: different lab may choose different elution buffer for your sample.
- For **Eluate Collection** (deck location 9): Label a 96-well Greiner Clear U-Bottom plate as “mAb Sample Plate”. Place the empty plate in deck location 9.

  Note: User can choose Greiner White U-Bottom plate if protease digestion will be used after affinity purification.

7. **CAUTION:** Make sure the labware on deck matches with what is shown in the software in the deck location. Placing the wrong labware will cause head crash.
8. **CAUTION:** Wiggle the labware in the plate pad to make sure that the labware is correctly seated within labware alignment features for each deck position. Warning: Labware positioned outside of lab alignment features will cause a head crash.
9. Remove any lid on the plate.
10. Click **Run Affinity Protection**.

Neutralize Sample

1. To neutralize the sample, navigate to **Utility Library**, and open **Reagent Transfer**.
2. Set the following parameters to transfer 5 µL of 5 % ammonium hydroxide to the sample (Figure 3).

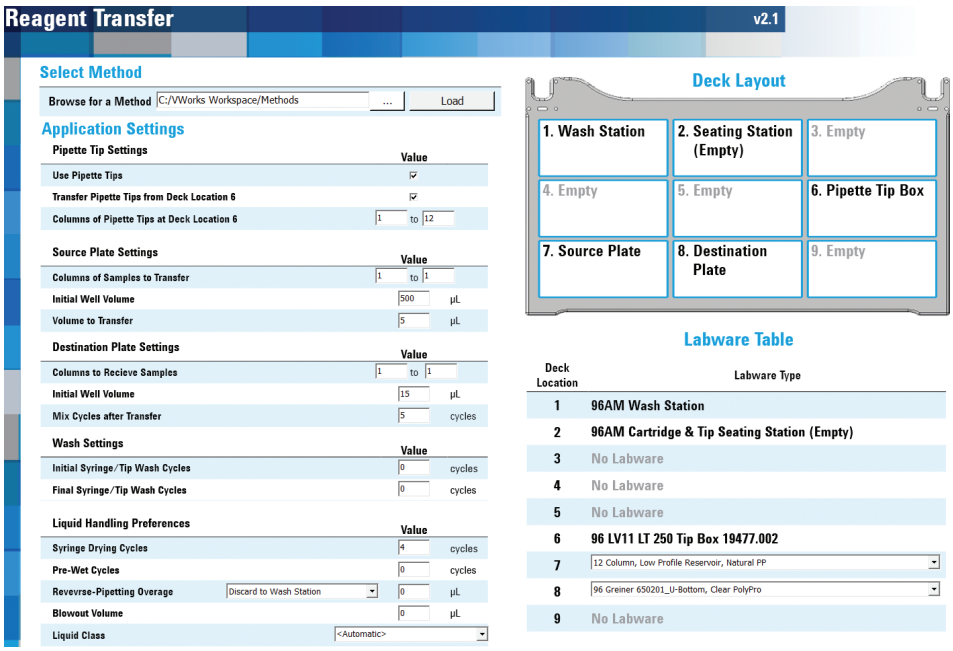

Figure 3. Reagent Transfer settings.

3. Transfer the following buffer and sample into its corresponding labware and place the labware in the deck location as indicated in the software interface.
    - Source Plate (deck location 7): Label a 12-well reservoir as “5 % Ammonium Hydroxide”. Manually fill the 1st column of the plate with **4 mL 5 % ammonium hydroxide**.

      Note: For the 12-column reservoir, the initial well volume was considered as 500 µL when adding 4 mL reagent to each column.
    - Destination Plate (deck location 8): Place the **Eluate Collection plate** from Affinity Purification.
4. Click **Run Protocol**.
5. Centrifuge the sample, seal the plate and keep at 4 °C until the next session.
6. Run **Shutdown**

Reference

1. Bovee, M.; Russell, J.; Murphy, S. Automation of Sample Preparation for Accurate and Scalable Quantification and Characterization of Biotherapeutic Proteins Using the Agilent AssayMAP Bravo Platform, *Agilent Technologies application note*, publication number 5991-4872EN, **2016**.

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