Protocol

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Rapid Antibody Digestion 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 v3.1** software using the desktop icon and select **Utility Library**.
2. Open the **Startup/Shutdown v2.0** utility by clicking on **Utility**.
3. Click **Run Startup** to run the startup utility with the default settings and read the notifications.

Denature, Alkylation, and Acidification

1. Navigate to **Workflow Library**, and open **Rapid Antibody Digestion Workflow**.
2. Under **In-Solution Digestion: Single Plate**, click **In-Solution Digestion**.

Note: 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 use one full column as an example here (Figure 1).

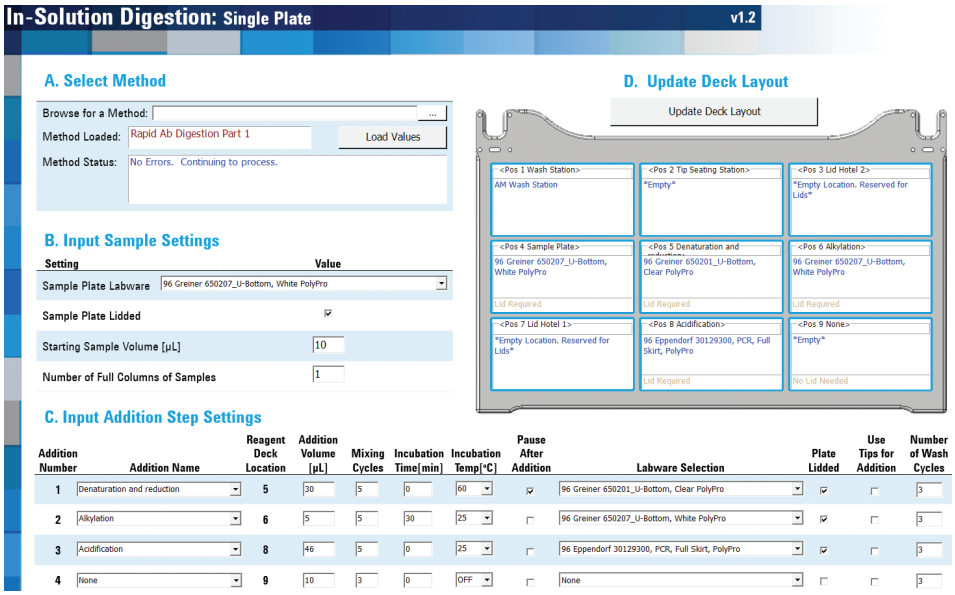


Figure 1. Single plate In-Solution Digestion screen.

3. Transfer the following buffer and sample into its corresponding labware, and place the labware on the deck location indicated in the software interface. Choose the labware in Figure 2.

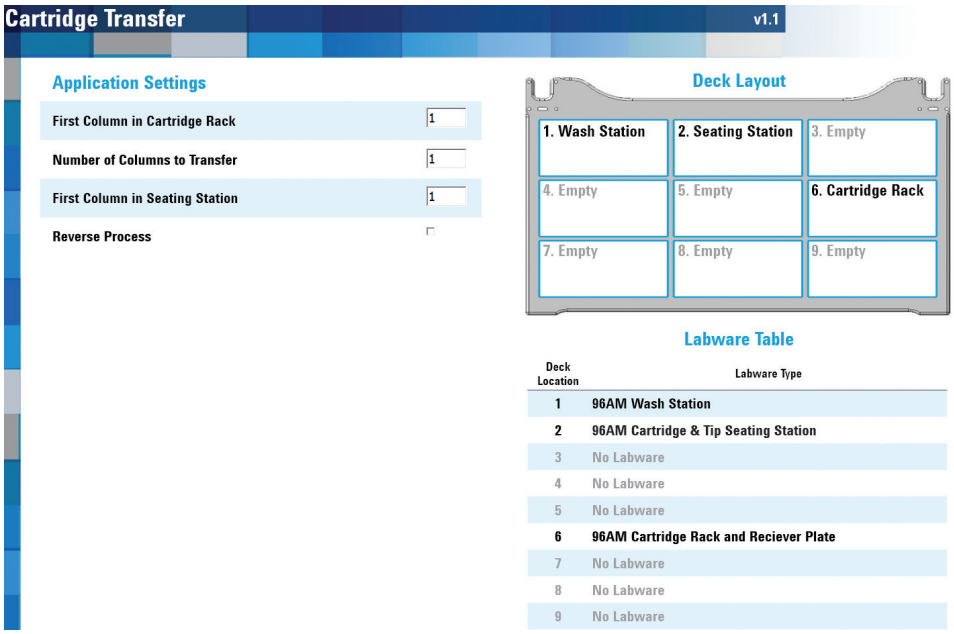


Figure 2. Cartridge Transfer parameters.

- **Sample** (deck location 4): Manually pipet 10 µL of the antibody samples (2.5 µg/µL) into the vials on plate. Cover the plate with a lid.

  Note: Consider two antibodies in use. Triplicate digestion for each sample. DI water can be used as a negative control.

	1	2
A	Sample 1	
B	Sample 1	
C	Sample 1	
D	Sample 2	
E	Sample 2	
F	Sample 2	
G	DI Water	
H	DI Water	

- **For denaturation and reduction** (deck location 5): Place the lidded denaturing/reducing buffer plate in place. (denaturing/reducing buffer content: 8 M guanidine, 0.5 M Tris, 13.3 mM TCEP, pH = 8.0).
- **For alkylation** (deck location 6): Place the lidded alkylation plate in place. (alkylant content: 100 mM iodoacetamide)

  Note: Alkylation needs dark conditions. The white plates and lids were in use.
- **For acidification** (deck location 8): Manually fill the 1st column of the plate with at least 56 µL 1.75% TFA and place in position.

  Note: Make sure the reagent loaded in each plate is the amount in use plus the dead volume of each plate type.

Note: Deck locations 3 and 7 were left empty and reserved for lids.

4. **CAUTION:** Make sure the labware on deck matches with what is shown in the software in deck Location. Labware mismatch will cause head crash. If the software has Plate Lidded checked, the plate on deck needs to have the lid on top.
5. **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 the labware alignment features will cause a head crash.
6. Click **Run Digestion**. When the program pauses for denaturation, remove the sample plate from deck location 4, seal the sample plate using the Agilent PlateLoc, and place it back to deck location 4 for incubation or in an incubator if needed. Start timer for one hour. After one hour, centrifuge the sample plate. Unseal the plate, and place it back at position 4. Put the lid on. Click **Continue**.

Note: Incubation can also be done on-deck if the user prefers a fully automated workflow without interruption by manual steps. Set incubation time to 60 minutes and uncheck **Pause After Addition**. The sample will be incubated at 60 °C with the lid on the plate.

Cartridge Transfer

1. Click **Workflow Library** and go back to **Rapid Antibody Digestion Workflow**. Choose **Cartridge Transfer**.
2. Set up the software as shown in Figure 2. Make sure the cartridge seating station is empty. Place the RP-W cartridge box at deck location 6.
3. Click **Run Protocol** to transfer one full column of cartridge to the cartridge seating station.

Protein Cleanup

1. Go back to **Rapid Antibody Digestion Workflow** and open **Protein Cleanup**.
2. Load the default setting for 5 µL of cartridges and click **Load** (Figure 3).

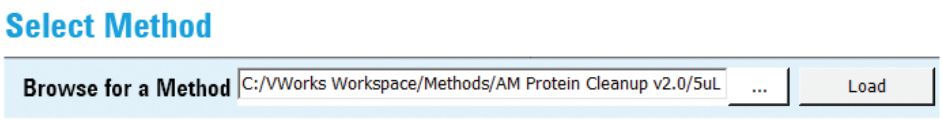


Figure 3. Select Method.

3. Set the **Protein Cleanup** parameters as shown in Figure 4.

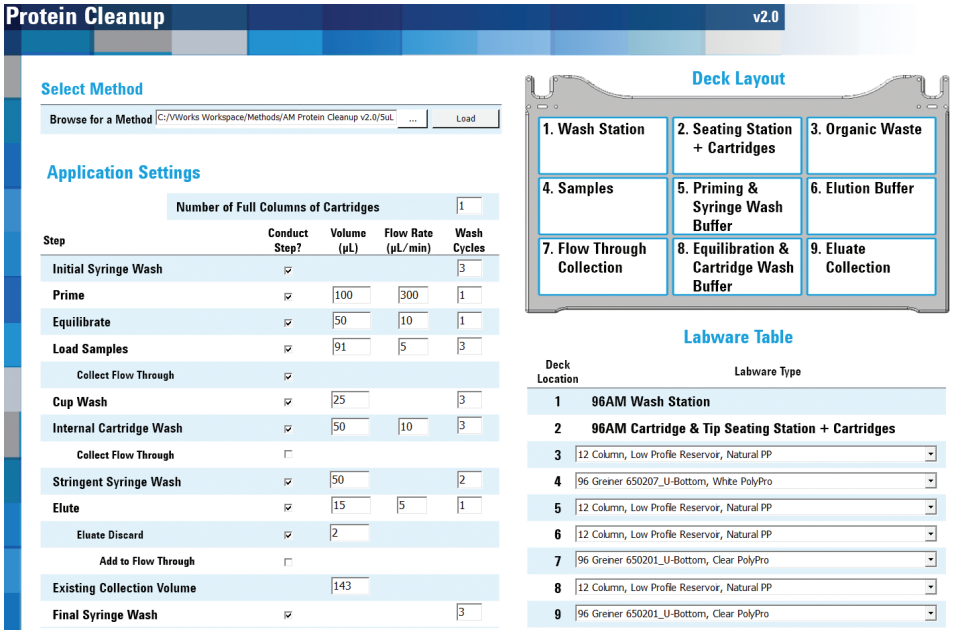


Figure 4. Protein Cleanup parameters.

4. Transfer the following buffer and sample into its corresponding labware and place the labware in the deck location as indicated in the software interface.
    - **Organic Waste** (deck location 3): Label a 12-column, low profile reservoir as "Organic Waste".
    - **Sample** (deck location 4): Place the denatured sample plate with samples in the first column.
    - **For Prime & Syringe Wash Buffer** (deck location 5): Label a 12-well reservoir as “Prime”. Manually fill the 1st column of the plate with 60 % acetonitrile, 0.1% TFA. Cover plate with a lid. (Lid will be removed before running the protocol).
    - **Elution Buffer** (deck location 6): Label a 12-well reservoir as “Elution”. Manually fill the 1st column of the plate with 70% acetonitrile, 50 mM ammonium bicarbonate, pH=8. Cover plate with a lid. (Lid will be removed before running the protocol).
    - **Flow Through Collection** (deck location 7): Label a 96-Greiner plate as “Flow through”. Place the labeled plate on deck location 7.
    - **Equilibration & Cartridge Wash Buffer** (deck location 8): Label a 12-well reservoir as “Equilibrate”. Manually fill the 1st column of the plate with 0.1% TFA.
    - **Eluate Collection** (deck location 9): Label a 96-well Greiner Clear U-Bottom plate as “mAb Sample Plate.” Manually fill the 1st column of vials with 143 µL 50 mM ammonium bicarbonate, pH=8.

Note: If the user wants higher recovery from a protein cleanup, elution using 60 to 70% acetonitrile, 0.1% TFA will be recommended. The sample can be dried in SpeedVac and reconstituted with digestion buffer, pH=8.

5. **CAUTION:** Make sure the labware on deck matches with what is shown in the software in deck location. A labware mismatch will cause a head crash.
6. **CAUTION:** Wiggle labware to make sure the labware is seated within labware alignment features for each deck position. Warning: Labware positioned outside lab alignment features will cause a head crash.
7. Remove any lid on the plate.
8. Click **Run Protocol**.

Protease Digestion

1. To add enzyme, navigate to **Utility Library**, and open **Reagent Transfer**.
2. Set the following parameters (Figure 5) to transfer 5 µL of enzyme to sample and mix.

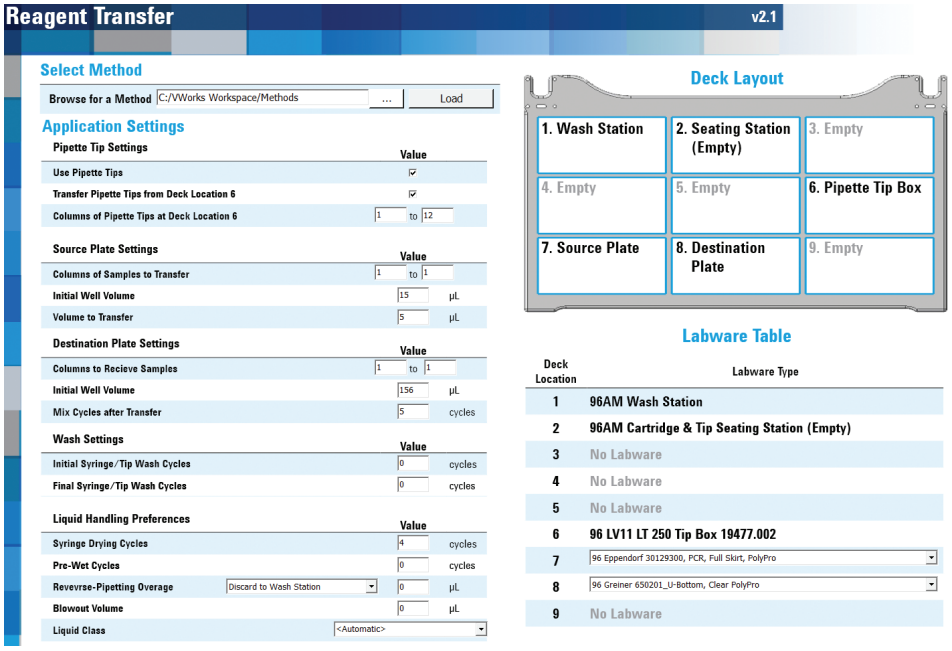


Figure 5. Reagent Transfer parameters.

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 96-well Eppendorf plate as “Enzyme”. Manually fill the 1st column of the plate with **15 µL enzyme**.
   - Destination Plate (deck location 8): Place the Eluate Collection plate from Protein Cleanup.
4. Click **Run Protocol**.
5. Centrifuge the sample, seal the plate using the Agilent PlateLoc, and incubate the plate at 37 °C for one or two hours for rapid digestion.

Reference

1. Bovee, M.; Krahenbuhl, A.; Murphy, S. Rapid Antibody Digestion Enabled by Automated Reversed-Phase Desalting, *Agilent Technologies application note*, publication number 5991-6478EN, **2016**.

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