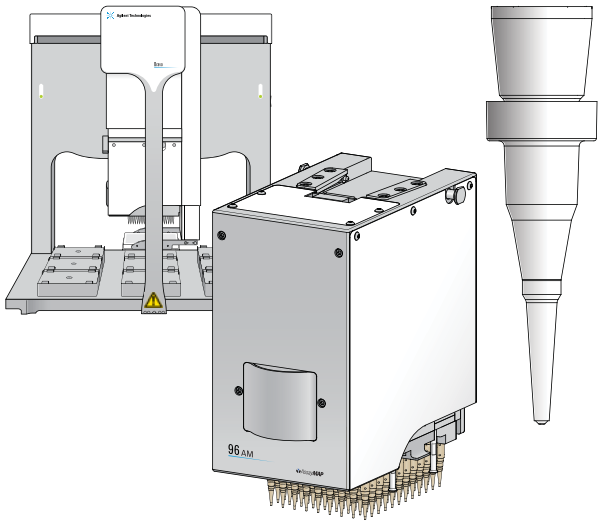
AssayMAP Bravo Protein A Purification Protocol Guide

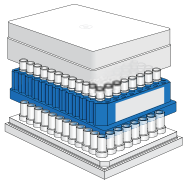
Overview

This guide describes a protocol for the purification of IgG antibodies on the Bravo Automated Liquid-Handling Platform using the Bravo 96AM Head with 96 PA-W AssayMAP® Bravo cartridges. For guidelines on using fewer than 96 channels and instructions on using the Bravo 96AM Head, see the *Bravo 96AM Head and AssayMAP Bravo Cartridges User Guide*.

Figure Bravo Platform, Bravo 96AM Head, and an AssayMAP Bravo cartridge



About the PA-W AssayMAP Bravo cartridges



The PA-W AssayMAP Bravo cartridges are packaged in a rack that holds 96 cartridges. The rack is covered with a lid and nests in a receiver plate. Each AssayMAP Bravo protein A cartridge contains a 5-µL bed packed with protein A resin. A proprietary coating protects the resin, stabilizing the surface chemistry so that the cartridges can be shipped dry. The cartridges require rehydration and equilibration before use. During the protein A purification protocol's priming and equilibration steps, the cartridges are rehydrated and the coating is removed.

Before you begin

Review the safety precautions in the *Bravo Automated Liquid Handling Platform Safety and Installation Guide*, and know how to stop in an emergency.

Ensure that the AssayMAP Bravo Platform has already been set up and that the device teachpoints have been verified for your configuration. For details, see the *Bravo Automated Liquid Handling Platform User Guide*.

Workflow

The protein A protocol contains all the steps for performing the purification, including preparing the AssayMAP Bravo cartridges for use, loading and binding the impure sample onto the selective resin in the cartridges, washing away the impurities from the bound IgG antibodies, and eluting the purified, concentrated IgG into a microplate for further processing or analysis.

Step	Description
1	Get the required equipment, materials, labware, and reagents
2	Prepare the cartridge receiver plate

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Step	Description
3	Open the VWorks protein A protocol form
4	Specify the parameter values
5	Set up the Bravo deck
6	Run the protein A protocol
7	Clean up after the run

Step 1—Get the required equipment, materials, labware, and reagents

Required equipment, materials, and labware

- *Bravo Platform with Bravo 96AM Head*. The Bravo Platform must have a gripper assembly and a computer running the VWorks software v11.1 or later. You use the VWorks software to run the protocol automatically. The VWorks protocol files (.pro) that this guide describes are available at: www.agilent.com/lifesciences/automation/ambravodownloads
- *Pump Module 2.0 and 96AM Tip Wash Station*. Ensure that the input tubing line (fill direction) between the Pump Module and the 96AM Tip Wash Station contains an inline filter.
- *Tips, if appropriate*. If your assay includes adding dye to the purified sample, use an Agilent 250-µL tip box with 96 250-µL LT tips.

  Note: To mount fewer than 96 tips (partial-head mode), the 96AM 250-µL Tip Loading Station is required to ensure proper seating of the tips on the head. See the *Bravo 96AM Head and AssayMAP Bravo Cartridges User Guide* for details.
- *Reservoirs and microplates*. You may select from the following labware options that are presented in the VWorks protocol form.

Labware manufacturer, part number, and name (VWorks form option)	Description
96-well manual fill reservoir, Agilent G5409A option 250, (**_ManualRes**)*	Recommended for the equilibration and elution buffer.
12-column low-profile reservoir, Axygen RESSW12LP, (**_ColumnRes**)	*Required for a partial head (columns).* For the equilibration and elution buffer. Fill the reservoir columns to correspond with the selected columns in the head.
8-row low-profile reservoir, Axygen RESSW8LP, (**_RowRes**)	*Required for a partial head (rows).* For the equilibration and elution buffer. Fill the reservoir rows to correspond with the selected rows in the head.
96-well microplate, Corning 3359, (**_96StdPlate**)*	Recommended for the unpurified sample and the Bradford dye. Alternatively, you can use one of the reservoir options.
96-well half-area microplate, Greiner 675801 (clear wells) or 67509*n* (clear well bottoms only) (**_HalfAreaPlate**)	Recommended for the eluate. If the eluate will be read in a spectrophotometer, for example, Bradford analysis, use a half-area microplate in the Greiner 67509*n* series. If you are performing A280 detection, use the Greiner 675801.

**Full-head option only.* This labware may be used with bare probes in full-head mode only. With mounted tips or cartridges, the same labware may be used with or without an *x-* or *y-*axis offset of the head from the deck location where the labware is located. For details, see the *Bravo 96AM Head with AssayMAP Bravo Cartridges User Guide*.

CAUTION If you use labware other than the options listed in the VWorks form, ensure that your labware is defined in the VWorks software and specified in the protocol. Using undefined labware on the Bravo deck can cause a crash resulting in damage to the Bravo 96AM Head.

Reagents

Ensure that you have a sufficient quantity of the reagents. For example, plan for the following quantities per 96-well assay:

- 1 L 1X Phosphate-Buffered Saline (PBS) pH 7–8, or other suitable wash buffer, to supply the 96AM Tip Wash Station and for equilibration
- 100 mL Elution Buffer (100 mM Glycine, pH 2.0)
- 25–100 µL (+25%) sample per well per assay
- *Optional.* 100 mL Coomassie Protein Reagent (Bradford reagent)
- *Optional.* IgG for constructing an analyte standard curve; either monoclonal antibody product or human IgG from serum
- Deionized water for rinsing the Bravo 96AM Head syringes before storage

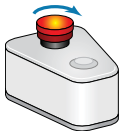
Step 2—Prepare the cartridge receiver plate

The cartridges are shipped dry and are rehydrated during the purification protocol. The following procedure fills the cartridge receiver plate with PBS buffer to help maintain the cartridge hydration state during any pauses in the protocol and during short-term storage.

CAUTION Hydrated cartridges that are left without the tips immersed in liquid for as little as 15 minutes can dry out, affecting performance. Discard any cartridge that dries out after the initial hydration. Rewetting dried cartridges is not recommended.

To fill the receiver plate wells with PBS buffer:

1. Verify that the Bravo Platform is ready to run. The indicator lights on the front of the device should be blue, and the robot-disable button should be activated.



2. In the **VWorks** window, choose **File > Open**, and select the AM utility protocol for the receiver plate: `AM_Utility_chargeReceiver.pro`

   If the Bravo device is not yet initialized, a message appears and asks if you would like to initialize the device.

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

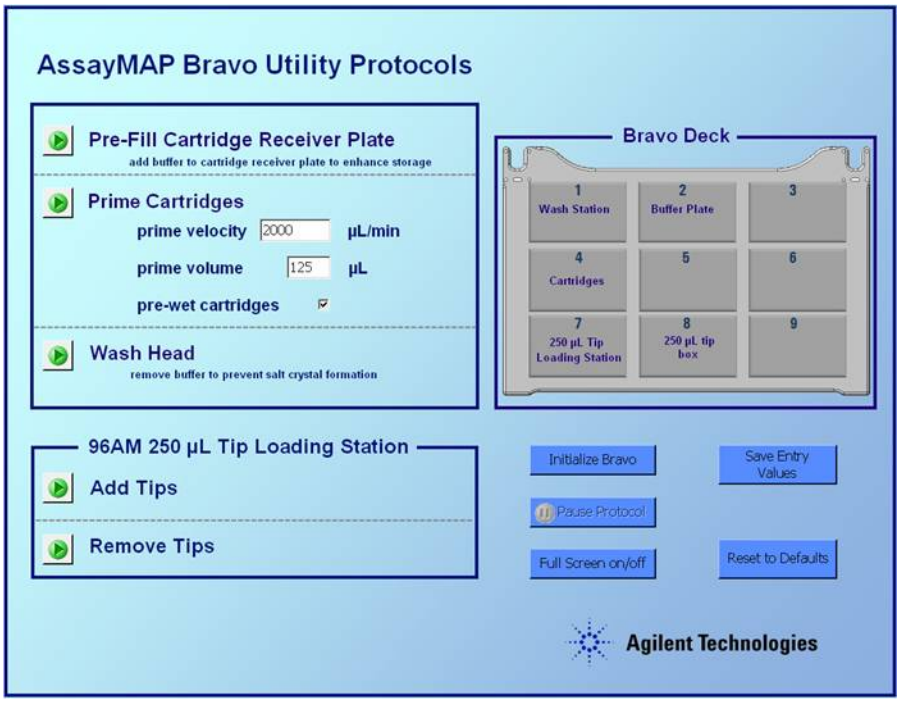
3. Click **Yes** to ensure that the Bravo Platform is initialized. Follow the instructions that appear on the screen.

   Note: If a fluid-in-tips message appears, but no fluid is in the tips, click Retry to continue homing the *w*-axis. If you click Ignore, you must home the *w*-axis before beginning a protocol.

   Note: If a microplate-in-gripper message appears, but the gripper is not holding labware, click Ignore to continue the homing process.

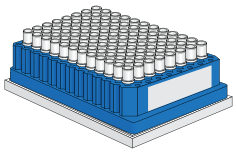
4 In the **AssayMAP Bravo Utility Protocols** form, click the run button ( ) next to **Pre-Fill Cartridge Receiver Plate** to start the utility protocol.

Figure VWorks form for AssayMAP Bravo Utility Protocols



5 Follow the on-screen instructions to position the receiver plate and a manual fill reservoir (or an inverted cartridge rack lid) that is filled with PBS buffer on the Bravo deck. A protocol-completed message appears when the protocol is finished.

6 When the Bravo robot has stopped moving, ensure that the cartridge rack is nested in the receiver plate and that the cartridges are seated securely in the rack, as the figure shows.



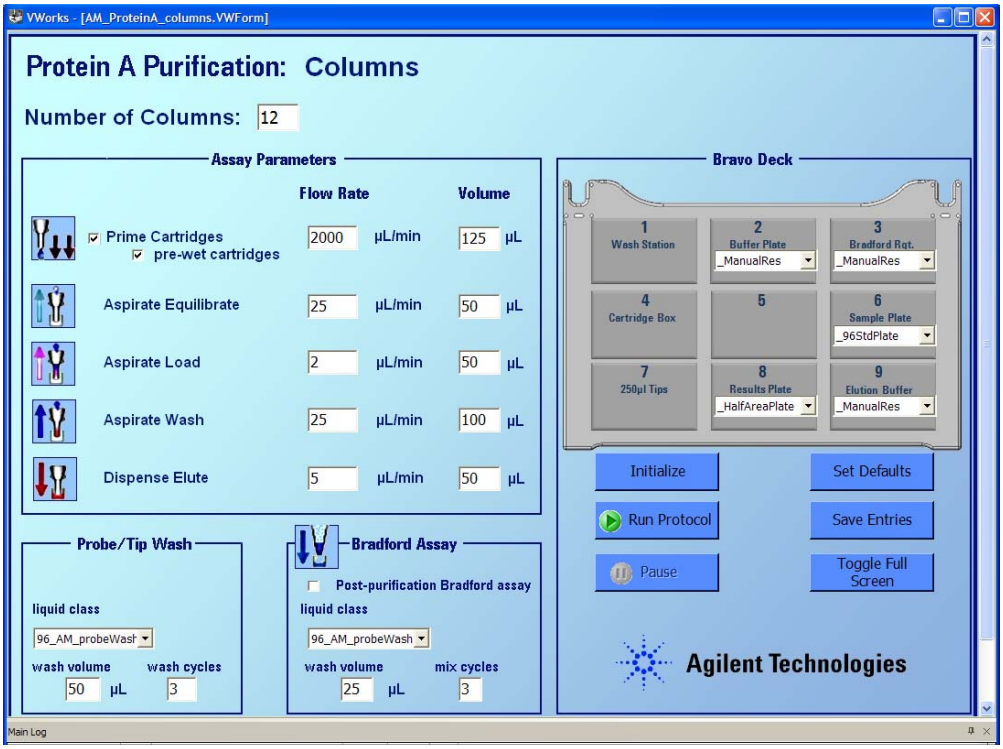
Step 3—Open the protein A protocol form

To open the protein A purification protocol in the VWorks software:

1 In the **VWorks** window, choose **File > Open**, and select the AM protein A purification protocol (.pro file) for the column- or row-format deck setup. The corresponding VWorks protocol form opens, as the following figure shows.

The column- and row-format protocols differ only in the deck setup. If you are using all 96 channels (full-head mode), you can use either the column- or row-based protocol. If you are using a subset of 96 channels (partial-head mode), choose the column- or row-based protocol, as appropriate, to match your channel selection in the Bravo 96AM Head. See the *Bravo 96AM Head and AssayMAP Bravo Cartridges User Guide* for additional partial-head liquid-handling requirements.

Figure VWorks form for AssayMAP Bravo protein-A purification protocol: Columns format



2 *Optional.* To enlarge the form, click **Toggle Full Screen**.

3 *Optional.* To refresh the form with default settings, click **Set Defaults**.

Step 4—Specify the parameter values

To specify the parameter values required for the protein A purification protocol:

1 Ensure the correct number of liquid-handling channels is specified in the **Number of Columns** or **Number of Rows** box.

2 In the **Assay Parameters** area, set the parameter options and specify the flow rate (µL/min) and volume (µL) for the following tasks:

Step		Description
	Prime Cartridges	Prepares the cartridges for first-time use by flushing a high volume of buffer through the cartridges at a fast flow rate to eliminate air bubbles and residual preservative from the resin.
	Pre-wet cartridges	*Optional.* Dispenses 10 µL of buffer just above the resin bed seal in the cartridge cup before mounting the cartridges to ensure that no air gets into the resin bed during the priming step.
	Aspirate Equilibrate	Adjusts the chemical conditions in the resin bed to be more conducive to binding or other functional steps, as necessary.
	Aspirate Load	Aspirates the unbound sample up through the mounted cartridges to bind the IgG antibodies to the resin in the cartridge bed. Use a flow rate that is slow enough to facilitate binding, yet fast enough so that the protocol runs at optimal speed.
	Aspirate Wash	Washes impurities from the resin in the cartridge bed so that only the cleaned, bound IgG antibodies remain in the cartridges.

Step		Description
	Dispense Elute	Dispenses elution buffer through the mounted cartridges to remove the bound IgG antibodies for sample collection.
	Bradford Assay	Uses disposable tips to transfer Bradford dye to the purified sample for further analysis.

3 In the **Probe/Tip Wash** area, select the liquid class, wash volume (µL), and number of cycles to use for the various washes throughout the protocol.

4 *Optional.* To add dye to the purified sample, select **Post-purification Bradford assay**, and then select the liquid class, volume, and number of mix cycles.

5 *Optional.* Click **Save Entries.** The next time the form is opened, the saved values appear.

Step 5— Set up the Bravo deck

The VWorks protocol form displays a top view of the deck showing the numbered locations and the labware for each location.

CAUTION The deck setup (column or row format) in the VWorks form must match the physical Bravo deck layout. Otherwise, a crash can occur between the Bravo 96AM Head and the items on the deck resulting in damage to the head.

To set up the Bravo deck:

1 In the **Bravo Deck** area of the VWorks form, select the labware that you are using for each location.

2 Place the labware on the Bravo deck so that the layout matches the **Bravo Deck** area on the VWorks form exactly. The following figures show the column- and row-format deck setups.

Figure Column-format deck setup: Bravo deck (left) and VWorks form (right)

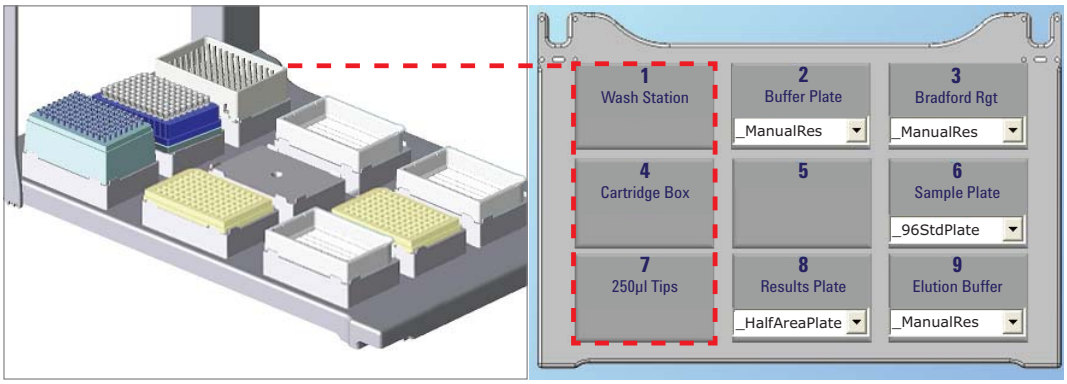
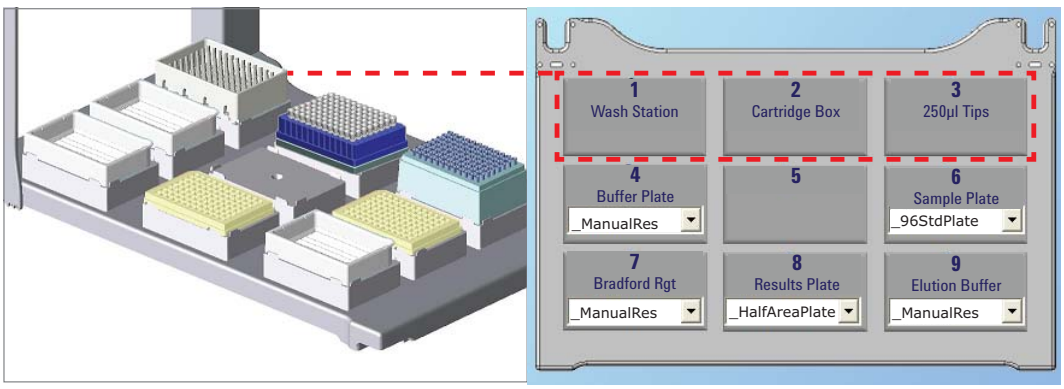


Figure Row-format deck setup: Bravo deck (left) and VWorks form (right)



Step 6—Run the protein A protocol

Before you begin, perform a dry run to check the new protocol and verify the device setup. A dry run allows you to troubleshoot a protocol without wasting valuable reagents and samples.

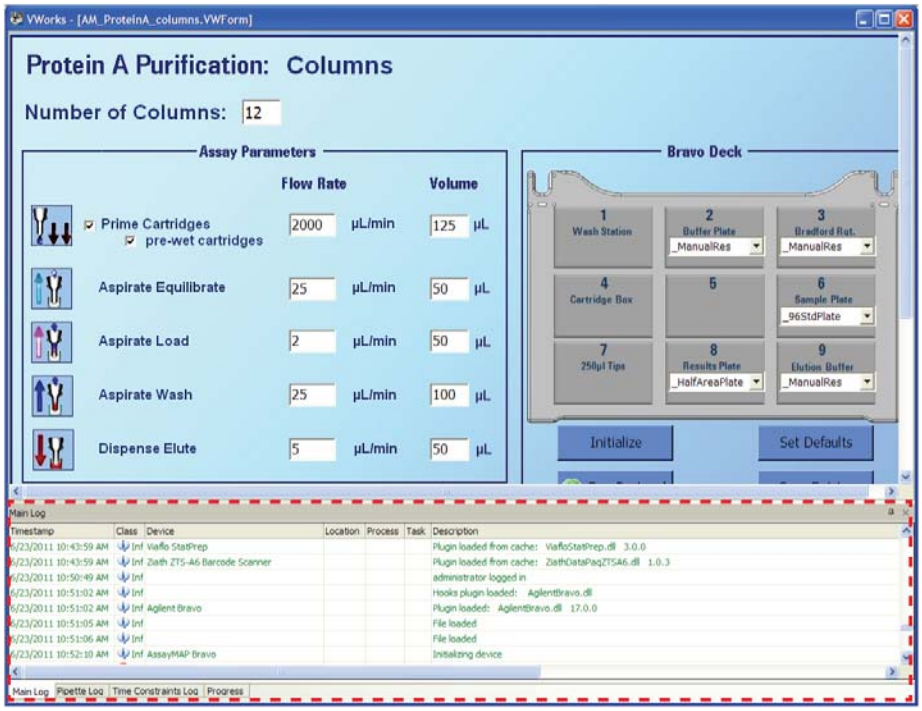
When you are ready to run the protocol with reagents:

- Fill the source bottle for the 96AM Tip Wash Station with sufficient PBS buffer, ensure the waste bottle is empty, and verify that the tubing connections are intact. The startup protocol steps will fill the Tip Wash Station automatically.
- Fill the microplates and reservoirs with the appropriate contents as specified by the protocol.
- For optimal performance, ensure the reagents have equilibrated to ambient temperature before you begin.

To run the protein A purification protocol:

1. In the **VWorks** form, click **Run Protocol** (Run Protocol).
2. To view the progress, including descriptions of each protocol step, use the horizontal splitter bar below the form to expand the **Main Log** area.

Figure VWorks Main Log area



Step 7—Clean up after the run

Reusing and storing the cartridges

After the initial hydration, the PA-W AssayMAP Bravo cartridges can be used multiple times. However, reuse is highly dependent on proper handling and storage, and the nature of the samples used on the cartridges.

CAUTION Avoid conditions during use and storage that can shorten the cartridge lifespan, including drying of the resin bed, accumulation of impurities and particulates, and microbial growth.

If you intend to reuse the cartridges, ensure that the cartridges do not dry out and that they are re-equilibrated in PBS buffer or a similar solution directly following the elution of the bound ligand. The VWorks protein A purification protocol, performs this re-equilibration automatically using the PBS buffer.

The hydrated cartridges can be stored in the cartridge rack, stacked on a receiver plate containing 200-µL per well of PBS buffer, and covered with the lid. You should validate the cartridge stability and performance on an individual basis to meet your requirements.

You may store unused cartridges as packaged at room temperature. Cartridges stored in this manner are stable for 6 months from the date of purchase.

Cleaning the Bravo 96AM Head before overnight storage

Use the following head wash procedure before leaving the Bravo 96AM Head idle overnight.

CAUTION To prevent salt buildup that could damage the seals, ensure that the Bravo 96AM Head syringes are washed thoroughly with deionized water before overnight storage.

To wash the Bravo 96AM Head syringes:

1. In the **VWorks** window, choose **File > Open**, and select the AM utility protocol for the head wash: `AM_Utility_head wash.pro`
2. In the **AssayMAP Bravo Utility Protocols** form, click the run button () next to **Wash Head** to start the protocol.
3. Follow the on-screen instructions to change the fluid in the Pump Module source bottle from buffer to deionized water.

   The protocol automatically empties the buffer from the Tip Wash Station, fills it with deionized water, and then performs the syringe washes.

   If you uninstall the head after cleaning the syringes, ensure that you use the storage stand provided. For details, see the *Bravo Automated Liquid Handling Platform User Guide*.

Getting more information

For information about...	See...
Care and handling of the Bravo 96AM Head and AssayMAP Bravo cartridges, and guidelines on creating protocols	*Bravo 96AM Head with AssayMAP Bravo Cartridges User Guide*
How to set up and maintain the Bravo Platform, and troubleshoot problems	*Bravo Automated Liquid Handling Platform User Guide*

You can access the product knowledge base from the VWorks software Help menu and online at: www.agilent.com/lifesciences/automation

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