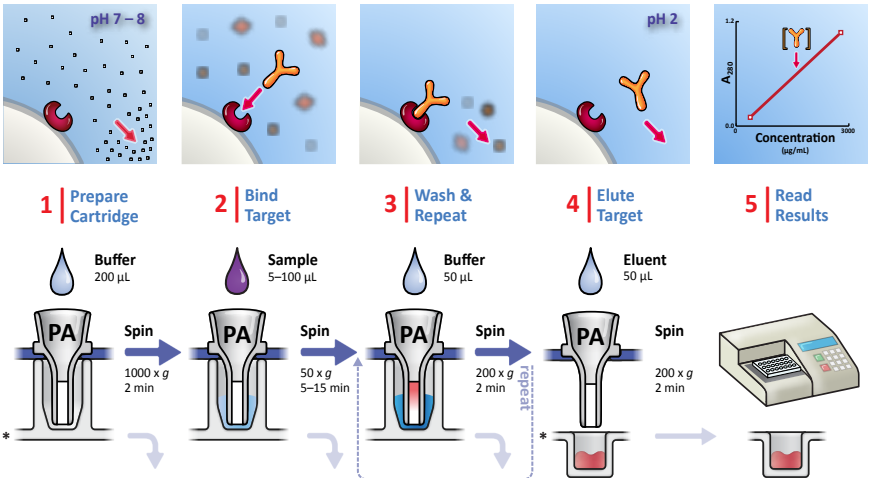


Protein A Cartridges Protocol

spin column format
for use with P50032, P50035, P50038



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a comprehensive guide to the
AssayMAP spin format technology

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AssayMAP® Protein A Cartridge Protocol (spin column format)

The AssayMAP Protein A cartridges offer a simple, precise, and high-throughput method for quantification of polyclonal or monoclonal antibody (MAb) titer in bioprocess samples. Using the same protein A chemistry employed in traditional affinity HPLC, the AssayMAP PA50 cartridges used in this assay offer rapid parallel sample processing with common laboratory equipment. All you need is an AssayMAP PA50 Starter Kit, a microplate centrifuge, common buffers, micropipettor, plate reader and this basic five-step protocol.

AssayMAP PA50 Starter Kit Contents (P50035)



- 24 reusable AssayMAP PA50 cartridges
- 2 empty AssayMAP Cartridge Racks
- 2 empty AssayMAP Receiver Plates
- 1 rack of Gilson Diamond® D200 pipet tips for loading samples

Cartridges contain a 5 μ L bed volume packed with a Protein A media (POROS® MabCapture® A, Life Technologies, Inc., Carlsbad, CA). The resin is protected by a proprietary coating that stabilizes the surface chemistry and allows the cartridges to be shipped dry. The cartridges require rehydration and equilibration before use. This coating is removed during the rehydration and equilibration step.

Also available: P50032 — AssayMAP PA50 Cartridges (96 reusable cartridges)
P50038 — AssayMAP PA50 24-Pack (24 reusable cartridges)

Storage and Use Conditions

Store unused cartridges at 4°C and use within 6 months of purchase.

Materials, Equipment, Labware and Reagents

- Microplate reader (capable of reading absorbance at 280 nm for direct protein concentration measurements and/or 450 and 590 nm for colorimetric protein concentration measurements)
- Micropipettor(s) for 10 – 200 μL volumes (multichannel pipet optional)
- Centrifuge (capable of 50 – 1000 $\times g$) and microplate rotor with a height capacity of 44 mm.
- Disposable pipet tips (recommend Gilson Diamond[®] Tips, D200, DL10)
- Results plate: Half-area flat-bottom SBS-compatible microplate. For A_{280} measurements, use a UV-transparent plate (e.g., Corning[®] P/N 3679); for A_{590}/A_{450} measurements (Bradford assay), use a standard transparent plate.
- Wash Buffer (PBS, pH 7 – 8 or other suitable wash buffer)
- Elution Buffer (recommend solutions tested in our lab, such as 100 mM Glycine, pH 2.0 or 12 mM HCl, 100 mM NaCl, pH 2.0; other solutions known to elute antibodies also should be compatible with PA50 cartridges.)
- Optional: Coomassie Protein Reagent (e.g., Thermo Scientific P/N 1856209)
- IgG for analyte standard curve: either monoclonal antibody product or human IgG from serum (e.g., Fluka P/N 56834)

Protocol

For optimal performance of AssayMAP PA50 cartridges, be sure to read the Best Practices section. All steps of this protocol should be performed at ambient temperature. Be sure that your reagents have equilibrated to ambient temperature before you begin.

1 | Prepare Cartridges: Rehydrate & Equilibrate

- a. To balance the rotor in subsequent centrifugation steps, distribute the cartridges you need to use equally across two empty Cartridge Racks and ensure that the cartridges are seated in the racks.
- b. Place the lid on the Cartridge Rack to prevent the cartridges from being pushed out when inserted into the Receiver Plate.
- c. Align the Cartridge Rack over the wells in the Receiver Plate, slide the rack onto the Receiver Plate and remove the lid.
- d. Pipet 200 μL of Wash Buffer into the cartridge sample cup.
Note: Do NOT introduce air into the cartridge while loading the sample. Read “Pipetting technique is critical”.
- e. Spin at 1000 $\times g$ for 2 minutes. Do not empty the flow through from Receiver Plate while you load samples on the cartridges.
Note: If you are repeating step 1 to rehydrate cartridges for reuse on the same day, stop here to keep the cartridge fully wet by leaving the Cartridge Rack on top of the Receiver Plate and the cartridge tips submerged in Wash Buffer.

2 | Load Cartridges: Bind Target

- a. Pipet 5-100 μL of sample into each cartridge sample cup using the recommended pipetting technique. Sample should have pH >6.0 and <8.5 and be clear of particulates.

Note: Do NOT introduce air into the cartridge while loading the sample. Read “Pipetting technique is critical” and “Understand the sensitivity range of the assay” in the Best Practices section before loading your sample.

- b. After samples are loaded, remove the Cartridge Racks from the Receiver Plates. Dump the Receiver Plate contents into a sink or waste container, and blot the tops with a paper towel.
- c. Place the cartridges back onto the receiver plate.
- d. Spin at $50 \times g$ until all of each sample is spun through its cartridge bed. The estimated spin time is 5 minutes for volumes between 5 and 25 μL or 15 minutes for volumes between 25 and 100 μL , but can be extended as needed. Refer to “Know the capabilities of your centrifuge” in the Best Practices for additional information.

3 | Wash Cartridges & Repeat: Remove Unbound Material

► First wash

- a. Pipet 50 μL Wash Buffer into each cartridge sample cup.
- b. Remove the Cartridge Racks from the Receiver Plates, dump the Receiver Plate contents into a sink or waste container and blot the tops with a paper towel.
- c. Place the cartridges back onto the receiver plate.
- d. Spin at $200 \times g$ for 2 minutes.

► Second wash

- a. Pipet 50 μL Wash Buffer into each cartridge sample cup.
- b. Remove the Cartridge Racks from the Receiver Plates, dump the Receiver Plate contents into a sink or waste container and blot the tops with a paper towel.
- c. Place the cartridges back onto the receiver plate.
- d. Spin at $200 \times g$ for 2 minutes.

4 | Elute Cartridges: Recover Purified MAb

- a. Pipet 50 μ L Elution Buffer into each cartridge sample cup.
- b. Place each rack on top of an appropriate Results Plate.
 - Use a UV-compatible, flat bottom half area plate for direct protein assay (A_{280}).
 - Use a standard transparent flat bottom half area plate for colorimetric assay (A_{590}/A_{450}).
- c. Spin at 200 x g for 2 minutes.

Note: If you plan to reuse the cartridges on the same day, be sure to keep the cartridge fully wet by repeating the rehydration step as soon as possible after elution and leaving it submerged in Wash Buffer (see Step 1 above)

5 | Measure Results: Read Absorbance

- ▶ For direct measurement of protein concentration:
 - a. Measure absorbance on plate reader at 280 nm.
- ▶ For colorimetric measurement of protein concentration (Bradford assay):
 - a. Add 125 μ L of Coomassie Reagent to each occupied well of the Results Plate.
 - b. Mix by pipetting.
 - c. Allow color to develop for 10 minutes.
 - d. Measure absorbance on plate reader at 590 and 450 nm and use the A_{590}/A_{450} ratio to calculate concentration by method of Zor and Selinsky (see “Understand the sensitivity range of the assay” in the Best Practices).

Best Practices

AssayMAP PA50 cartridges are reusable.

AssayMAP protein A cartridges are prepared using a proprietary method that allows the cartridges to be shipped and stored dry. After rehydration, the AssayMAP cartridges can be reused 5 to 10 times or more provided they are kept wet and handled properly. Multiple runs performed in a single day should present no problems. Avoid conditions during use and storage that can shorten cartridge lifespan, including drying of the resin bed, accumulation of impurities and/or particulates and microbial growth.

Prior to short term storage, hydrated cartridges should be re-equilibrated in equilibration buffer and then stored at 4°C in racks with lids stacked on top of receiver plates containing the same equilibration buffer. Wet cartridges stored in this manner should be stable for at least one week in the absence of

microbial growth, but should be validated on an individual basis to meet your requirements.

Pipetting technique is critical.

The accuracy and precision of the AssayMAP PA50 assay is critically dependent upon the following pipetting techniques:

► Loading steps - pipetting samples

Sample loading steps are always preceded by steps that result in 200 μL in the receiver plate and a small volume of liquid on top of the resin bed. This liquid provides the means, along with the pipetting loading technique described below, to load the sample with a liquid-to-liquid interface. This is designed to prevent entrapment of air bubbles which can prevent proper flow. All sample loading steps include centrifugation at 50 x g , which may not provide sufficient force to break bubbles that can enter into the resin.

Using a Gilson Diamond 200 tip with graduation ridges, load your sample by inserting the pipet tip into the cartridge at such an angle that the tip of the pipet runs along the inside surface of the cartridge and the other side of the pipet tip slides along the rim of the cup until the 20 μL graduation ridge of the pipet tip is resting against the top edge of the cartridge. Gently depress the plunger of the pipet to the first stop to dispense the desired volume. Do NOT depress the plunger to the second stop yet, which uses an additional volume of air to blow out any solution remaining in the tip and can introduce an air bubble in the throat of the cartridge near the top of the resin bed. Lift the pipet tip straight up. Touch the tip of the pipet to the top of the sample cup then depress to the second stop. This will ensure that all sample is dispensed into the sample cup.

► Other steps - pipetting buffers

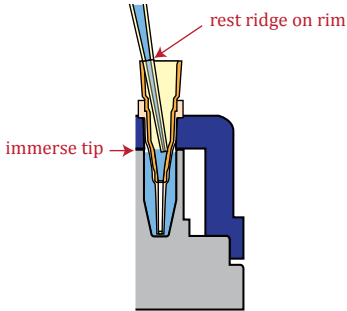
To dispense buffers used in equilibration, washing and elution steps, place the pipet tip against the inside edge of the top section of the cartridge sample cup. Gently depress the plunger to the first stop. Lift the pipet tip straight up. Touch the tip of the pipet to the top of the sample cup then depress to the second stop.

► Airlock

If a cartridge is observed to not flow, the cause is most likely an airlock. DO NOT EMPTY the receiver plate for any cartridges. The bubble causing the airlock can be easily displaced by setting your pipet to approximately half the volume that is in the cartridge, pushing the plunger down to the first stop and then putting the pipette tip into the cartridge using the “loading steps - pipetting sample” to place the pipet tip in the cartridge. Pipet up and down to displace the bubble taking care not to introduce another bubble. Repeat the spin that led to the airlock (other cartridges without airlocks can be spun in parallel). This manipulation and supplemental spin will not affect the results of the experiment.

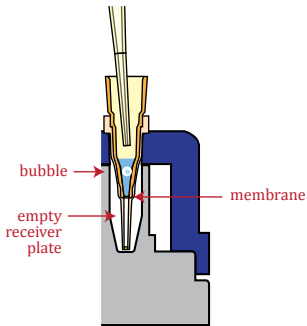
Sample Loading

Use this technique for each sample loading step that precedes a 50 x g spin.



✓ Yes

DO rest the 20 μ L graduation ridge on cartridge rim, just immerse tip into liquid in the throat of the sample cup after the rewet/equilibration step and depress pipet to first stop only. Also see "Touching Off" for more info.

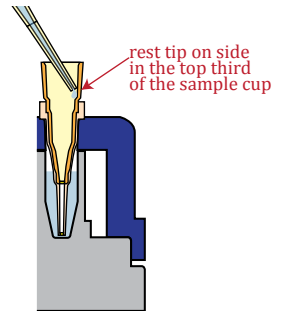


✗ No

DO NOT load sample without liquid in the receiver plate, touch the membrane at the top of the resin bed, pipet too quickly or introduce bubbles that can cause an airlock.

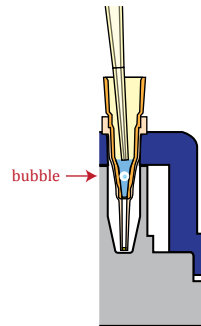
General Pipetting

Use this technique for pipetting equilibration, wash and elution liquids preceding a 200 x g or faster spin.



✓ Yes

DO rest the pipet tip inside the sample cup (above the rack), let liquid flow down the side of the sample cup, and depress pipet only to the first stop. Also see "Touching Off" for more info.

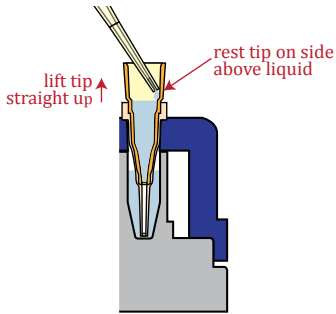


✗ No

DO NOT position tip incorrectly, depress pipet past first stop while submerged, pipet too quickly or introduce bubbles that can cause an airlock.

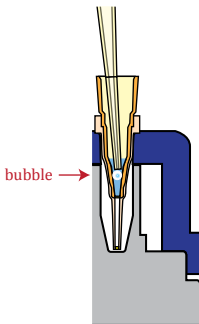
Touching Off

Use this technique for blowing out any liquid that remains in a pipet tip after depressing the pipet to the first stop.



✓ Yes

DO rest the pipet tip inside the sample cup above the liquid, depress pipet to the second stop and lift the pipet tip straight up when complete.

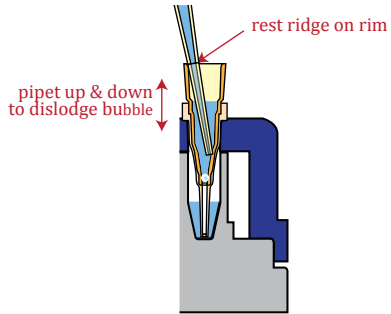


✗ No

DO NOT depress pipet past first stop while submerged, which can introduce bubbles that can cause an airlock.

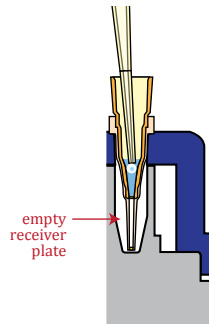
Troubleshooting Airlocks

Use this technique for a cartridge containing a bubble in the sample cup before centrifugation or for a cartridge that still contains liquid after centrifugation (suspected airlock).



✓ Yes

DO set your pipettor to approximately half the volume in the sample cup, rest the 20 μ L graduation ridge on cartridge rim, depress plunger to the first stop, pipet in and out to dislodge bubble and respin set of cartridges, if necessary.



✗ No

DO NOT introduce additional bubbles or remove liquid from the receiver plate before this step.

Know the capabilities of your centrifuge.

Microplate centrifuges vary significantly. The “ $x g$ ” values and spin times recommended in this protocol are generally sufficient for most centrifuges. However, it is prudent upon first use to make sure that all the cartridges are completely empty after each protocol step by visually inspecting from the top and the side of the cartridge. When viewed from above, a cartridge containing liquid will show a reflective liquid surface rather than a matte resin bed surface. When viewed from the side, a cartridge containing liquid will show a visible meniscus above the top of the resin bed. Some upward adjustment of spin time may be necessary to ensure that every cartridge has emptied after each step. The rotor must be able to accommodate cartridges seated in the Cartridge Rack and Receiver Plate assembly (44 mm height capacity).

Keep the cartridge wet.

Proper hydration of the cartridge is critical for good performance. New cartridges are shipped dry in a special preservative that preserves binding capacity. Step 1 in the protocol rehydrates the cartridges and removes all air from the bed. After you have rehydrated your cartridges, proceed with the protocol until completion, and if reuse is planned, be sure to repeat protocol Step 1 as soon as possible after the elution step. Hydrated cartridges left on the lab bench for as little as 15 minutes may dry out, affecting performance. Cartridges may be stored for longer periods immersed in the Receiver Plates used for rehydration (protocol Step 1). Also see: “AssayMAP PA50 cartridges are reusable” in Best Practices.

Warning: *If a cartridge dries out after the initial hydration, it should be discarded. Rewetting dried cartridges is not recommended.*

Balance your centrifuge rotor.

When preparing the Cartridge Racks for inserting onto the plate carriers in your centrifuge, distribute experimental samples equally across two racks by matching volumes, cartridge, rack positions and receptacles (Receiver Plates or Results Plates). For example, if you’re running 16 samples, place 8 cartridges on rack A and 8 cartridges on rack B in the same relative positions.

Utilize the sample volume capacity of the cartridge.

Sample volumes ranging from 5 to 100 μL can be loaded into the cartridge reservoir, easily allowing analysis of a broad range of sample concentrations without the need for sample dilution. If necessary, you can apply dilute samples in multiple loads. For example, you can apply 100 μL , spin, apply another 100 μL to the same cartridge, and then continue with the protocol.

Understand the sensitivity range of the assay.

The range of the Protein A assay is based on the mass of the MAb (μg) captured on the cartridge. The assay has a practical upper limit defined by the

quantitative binding capacity of the cartridge, which is approximately 100 µg IgG. Lower detection and quantitation limits are a consequence of your plate reader and choice of detection method (A_{280} and/or colorimetric), as well as the effectiveness of the wash step to eliminate non-specific binding of the given sample matrix.

Since your goal is generally to measure concentration, you will need to consider your samples in terms of mass (Mass = Concentration x Volume) to choose an appropriate sample volume to produce results within the required assay range. You can use multiple cartridges to test a range of sample volumes (for example, 10, 30 and 100 µL) without propagating sample dilution errors. You can also expand the range for a given sample volume by first measuring the A_{280} of the result plate, then adding protein assay reagent and making a second colorimetric measurement. For many applications in cell culture process development, a sample volume of 25 µL provides a useful concentration range (100 – 4000 µg/mL) using A_{280} detection.

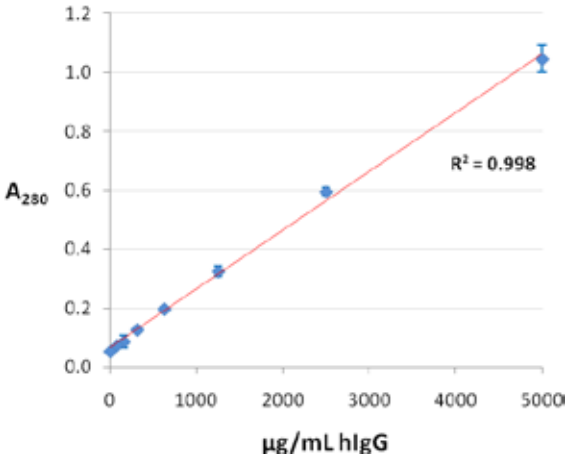
Detection Method	Limit of Detection	Upper Limit
A_{280}	1 µg	100 µg ^A
Colorimetric	0.2 µg	20 µg ^B

- A. The upper limit for A_{280} is determined by the mass of IgG the cartridge can quantitatively capture during sample loading. Measurements can be made above this limit, but are much more subject to variability and require non-linear curve fitting.
- B. This expanded upper limit is for the Bradford assay using the method of Zor and Selinger (*Anal Biochem* 236:302-308 1996), which compares the absorbance ratio (A_{590}/A_{450}) to a set of standards.

Use appropriate standards.

For best interpolation of results for unknowns, create a set of standards covering the range of expected antibody concentrations and run these standards along with your unknown samples through all the steps of the protocol. Use the results from the standards to create a standard curve used to determine concentrations of unknowns. Because antibodies differ in extinction coefficient or response in colorimetric assays, ideally one should use the antibody in the unknowns if at all possible for the standards.

Standard Curve



Typical standard curve using the AssayMAP PA50 cartridges with 25 μL samples of polyclonal human IgG in PBS and A_{280} quantitation. Data plotted are the means of 6 replicates \pm standard deviation.

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