I. Introduction

This protocol is provided for **Capturem Protein A 96-Well Plate** (Cat. No. 635716), a single-use disposable 96-well plate for simple, rapid purification of antibodies from animal sera, ascites fluid, cell culture media, and other sources. Each well can hold up to 1 ml of sample and requires a minimum elution volume of 100 µl. The binding capacity varies, depending upon the sample type, species, and antibody isotype. More information about bed volume and capacities can be found on our website.

II. Materials and Reagents

A. Components

- 1 Capturem Protein A 96-Well Plate

B. Additional Materials Required

1. Purification Buffers

This kit is compatible with all standard buffers typically used for antibody purification, such as phosphate, citrate, and glycine-based buffers. We highly recommend using a binding buffer with an optimal pH ~8 (preferably Protein A IgG Binding Buffer from Thermo Fisher Scientific, Cat. No. 21001). Loading the sample (e.g., serum matrix) without prior dilution is not recommended.

- **Equilibration/Binding/Wash Buffer:** To obtain optimal and reproducible performance, we recommend using Pierce Protein A IgG Binding Buffer (Thermo Fisher Scientific). Alternatively, 0.1–0.5 M phosphate containing 0.5–2 M NaCl, pH 8.0, or 1 M glycine containing 2 M NaCl, pH 9.0 may be used. Depending on the antibody subtype, the yield and purity may vary according to the salt content and pH of these buffers. Do not use PBS buffer.
- **Elution Buffer:** 0.1 M glycine, pH 2.5–3.0
- **Neutralization Buffer:** 1 M Tris, pH 8.5

2. Collection Plates

- **96-well collection plates:** Each purification will require four standard 96-well collection plates. These plates should be used throughout the protocol to collect flowthrough and wash samples that will be saved for SDS-PAGE analysis and/or colorimetric protein assays (e.g., Bradford assays). Alternatively, if only the eluates are to be analyzed, the flowthrough from the equilibration and wash steps can be discarded. In this case only one collection plate is required, for collecting the eluate.
- **96-well plate filtration device:** The Capturem Protein A 96-Well Plate is compatible with standard 96-well plate filtration devices, such as the NucleoVac 96 Vacuum Manifold (Cat. No. 740681).
- **96-well plate centrifuge:** The Capturem Protein A 96-Well Plate is compatible with standard 96-well plate centrifuges (e.g., Eppendorf Centrifuge 5804 R with Deepwell-plate rotor, A-2-DWP).
- **Multi-channel pipette**

III. Sample Preparation

1. Follow the standard protocols for preparing antibody samples. We recommend diluting the antibody sample in the range of 1:1–1:15 sample in a binding buffer like Protein A IgG Binding Buffer from Thermo Fisher Scientific. We recommend a 1:1–1:4 dilution with binding buffer for hybridoma samples, and a 1:15 dilution with binding buffer for serum samples, which may differ depending on the animal species and may require further optimization for obtaining high yield and purity. **Loading the sample (e.g., serum matrix) without prior dilution is not recommended.**
2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto a spin column.

IV. Antibody Purification

Purification of antibodies from the samples prepared in Section III may be performed using vacuum filtration (Protocol A) or centrifugation in a standard 96-well plate centrifuge (Protocol B).

A. Protocol: Antibody Purification Using Vacuum Filtration

1. Assemble the Capturem Protein A 96-Well Plate in a 96-well plate filtration device together with a 96-well collection plate (supplied by the user—see Section II.B).
2. **Equilibrate:** Add 800 µl Equilibration Buffer (preferably Protein A IgG Binding Buffer from Thermo Fisher Scientific) to each well of the Capturem Protein A 96-Well Plate using a multi-channel pipette to equilibrate the wells. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. Remove the flowthrough and discard it along with the collection plate. Reassemble the Capturem Protein A 96-Well Plate in the 96-well plate filtration device together with a new collection plate.

   NOTE: The required pressure may vary between 0.2–0.8 bar, depending on the sample. When performing vacuum filtration, use the following formula to convert between different vacuum pressure units:
   Pressure Conversion Formula:
   1 mbar = 100 Pa = 0.750 mm Hg = 14.504 x 10^{-3} psi = 0.987 x 10^{-3} atm
3. **Bind:** Load 200–1,000 µl diluted antibody (from Section III) onto each well of the equilibrated Capturem Protein A 96-Well Plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis. Reassemble the Capturem Protein A 96-Well Plate in the 96-well plate filtration device together with a new collection plate.
4. **Wash:** Add 800 µl Wash Buffer to each well of the Capturem Protein A 96-Well Plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.
5. **Elute** your antibody from the Capturem Protein A 96-Well Plate as follows:
   a. Add 10–30 µl Neutralization Buffer (1/10 the volume of Elution Buffer to be used) to each well of a new collection plate and reassemble the collection plate together with the Capturem plate in the 96-well plate filtration device.
   b. Add 100–300 µl Elution Buffer to each well of the Capturem Protein A 96-Well Plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the Capturem plate wells into the collection plate. The wells of the collection plate should contain your eluted tagged protein, which is now ready for analysis.

NOTE: ≥90% of your antibody can be eluted with 100 µl of Elution Buffer.

6. Measure the amount of antibody in your flowthrough samples from Steps 3 and 4, and your eluate(s) from Step 5, using absorbance at 280 nm or another colorimetric protein analysis method. One OD_{280} unit typically corresponds to 0.73 mg/ml of IgG. The purity of the eluted antibodies can be analyzed by SDS-PAGE, size exclusion chromatography, etc.

B. Protocol: Antibody Purification Using Centrifugation

1. Place the Capturem Protein A 96-Well Plate securely on top of a 96-well collection plate (supplied by the user—see Section II.B).
2. **Equilibrate:** Add 800 µl Equilibration Buffer, preferably Protein A IgG Binding Buffer from Thermo Fisher Scientific, to each well of the Capturem Protein A 96-Well Plate using a multi-channel pipette to equilibrate the wells. Centrifuge at 2,000*g* for 3 min at room temperature. Remove the flowthrough and discard it along with the collection plate. Place the Capturem Protein A 96-Well Plate securely on top of a new 96-well collection plate.

   NOTE: When performing centrifugation, use the following formula to convert rpm to centrifugal force units (RCF or g) if the centrifuge does not automatically provide this information:

   Centrifugal Force Conversion Formula:
   $RCF \text{ or } g = (1.12) \times (R) \times (rpm/1000)^2$

   where R is the distance (in millimeters) from the center of the rotor to the end of the spin bucket when held horizontally and away from the center.

   If the wells do not drain completely, centrifuge again at 2,000*g* for 3 min.
3. **Bind:** Load 200–1,000 µl diluted antibody (from Section III) onto each well of the equilibrated Capturem Protein A 96-Well Plate. Centrifuge at 2,000*g* for 3 min at room temperature. Save the collection plate containing the flowthrough for protein analysis. Place the Capturem Protein A 96-Well Plate securely on top of a new 96-well collection plate.
4. **Wash:** Add 800 µl Wash Buffer to each well of the Capturem Protein A 96-Well Plate. Centrifuge at 2,000*g* for 3 min at room temperature. Save the collection plate containing the flowthrough for protein analysis.
5. **Elute** your antibody from the Capturem Protein A 96-Well Plate as follows:
   a. Add 10–30 µl Neutralization Buffer (1/10 the volume of Elution Buffer to be used) to each well of a new collection plate and place the Capturem plate securely on top of the collection plate.
   b. Add 100–300 µl Elution Buffer to each well of the Capturem Protein A 96-Well Plate. Centrifuge at 2,000*g* for 3 min at room temperature. The wells of the collection plate should contain your eluted tagged protein, which is now ready for analysis.

   NOTE: ≥90% of your tagged protein can be eluted with 100 µl of Elution Buffer.
6. Measure the amount of antibody in your flowthrough samples from Steps 3 and 4, and your eluate(s) from Step 5, using absorbance at 280 nm or another colorimetric protein analysis method. One OD_{280} unit typically corresponds to 0.73 mg/ml of IgG. The purity of the eluted antibodies can be analyzed by SDS-PAGE, size exclusion chromatography, etc.

Appendix A. Troubleshooting Guide

Problem	Possible Explanation	Solution
Background bands/low purity	Nonspecific binding of proteins to the membrane.	Add an additional wash step with Wash Buffer after Step 4 of Section IV.B.
Low percentage recovery	The sample contains more antibody than the Capturem Protein A 96 Plate has the capacity to bind.	Reduce the amount of sample added to each well. If you need to purify more antibody, consider using the Capturem Protein A Maxi kit, which has a higher binding capacity (1–2 mg) or the Capturem Protein A 24-Well Plate.
Low yield of purified antibody	Poor binding affinity for the IgG subtype used. Different subtype classes and species bind differently to Protein G. Please, refer to *J. Chromatogr A.* **2007**,1160, 44-55.	Verify that Protein A-based purification is suitable for the IgG subtype class and species used. Alternatively, consider using Protein G-based purification instead.
	The pH of the binding buffer was altered by the hybridoma, serum, or cell culture medium used.	Make sure the pH of the buffer is in optimal range (pH 8–9). We recommend Protein A IgG buffer from Thermo Fisher Scientific as binding buffer.
	Suboptimal dilution	Make sure to use the optimal dilution for your sample. For hybridoma medium, a 1:2 to 1:4 dilution was found to work well, depending on the sample.
Antibody does not elute	Elution conditions are too mild	• Follow the instructions using the recommended elution buffer (0.1 M glycine, pH 2.5–3.0) • Make sure the pH of the elution buffer is 2.5–3.0 if you are using a different buffer than the one recommended. • Add an additional elution step as described in Step 5 of Section IV.B.
Spin column does not fully drain	Clogging due to particles or very viscous sample	• Pre-clarify the solution by centrifugation or using a 0.8-micron filter • Repeat centrifugation at 2,000*g* for 3 min.

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