I. Introduction

This protocol is provided for the **Capturem Protein A 24-Well Plate - HC** (Cat. Nos. 635742 & 635743), a single-use, disposable 24-well plate for the simple, rapid purification of antibodies from animal sera, ascites fluid, cell culture media, and other sources. Each well can hold up to 4.5 ml of sample and requires a minimum elution volume of 500 µl. Depending upon the sample type, species, and antibody isotype being purified, the binding capacity of the Capturem membranes will vary. More information about bed volume and capacities can be found on our website.

II. Materials and Reagents

A. Components

Cat. No. 635742:

- 4 x Capturem Protein A 24-Well Plate - HC

Cat. No. 635743:

- 1 x Capturem Protein A 24-Well Plate - HC

B. Additional Materials Required

1. Purification Buffers

This kit is compatible with all standard buffers typically used for antibody purification, such as phosphate- and acetate-based buffers. Loading serum samples without prior dilution is not recommended. Depending on sample, transiently transfected cell supernatants (e.g., Expi293 HEK cells) may be loaded without prior dilution in binding buffer.

- **Equilibration/Binding/Wash Buffer:** We recommend using Takara Bio's 10X Capturem Protein A Buffer (Cat. No. 635746). The Capturem Protein A Buffer should be diluted to 1X before use. Alternatively, we have found that the following buffer compositions also work with Capturem Protein A 24-Well Plate - HC:
  - 0.1 M Tris, 150 mM NaCl (pH 8.0), 100 mM phosphate
  - 2 M NaCl (pH 8.0), 100 mM sodium phosphate (pH 6.8)
  - 2.5 M NaCl (pH 7.4), 100 mM sodium citrate (pH 7.4)
  - Dulbecco's PBS
- **Elution Buffer:** 0.1 M glycine (pH 2.5–3.0) or 0.1 M citric acid (pH 2.5–3.0)
- **Neutralization Buffer:** 1 M Tris (pH 8.5)

2. Collection Plates

- **24-well collection plates:** Each purification will require four standard 24-well collection plates (e.g., Thomas Scientific, Cat. No. 1149Q53, 24-Well Polypropylene Storage/Reaction Microplate, 10mL/square well, round bottoms, 44mm height). These plates should be used throughout the protocol to collect flowthrough, wash, and elution 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 two collection plates are required, one for collecting the flowthrough and washes and a second for the actual eluates.

- **Recommended filtration device:** The Capturem Protein A 24-Well Plate - HC is compatible with standard 96-well plate filtration devices, such as the NucleoVac 96 Vacuum Manifold (Cat. No. 740681).
- **Recommended plate centrifuge:** The Capturem Protein A 24-Well Plate - HC is compatible with standard plate centrifuges (e.g., Eppendorf Centrifuge 5804 R with deepwell-plate rotor, A-2-DWP).
- **Recommended positive pressure device:** The Capturem Protein A 24-Well Plate - HC is compatible with standard 96-well positive pressure devices (e.g., Waters Positive Pressure-96 Processor, Cat. No. 186006961).
- **Single-channel pipettes** (various).

III. Sample Preparation

1. Follow the standard protocols for preparing antibody samples. We recommend diluting the antibody sample in the range of 1:2–1:50 sample to Capturem Protein A Buffer. We recommend a 1:2–1:4 dilution for hybridoma samples; and a 1:15 dilution for serum samples, which may differ depending on 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. Loading high-density cell-culture supernatants (e.g., Expi293 HEK cells) directly without dilution may work from case to case but could lead to higher well-to-well variability.

   NOTE: The Capturem Protein A Buffer has a 10X concentration. Remember to dilute it to 1X before use.

2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.5-micron filter before loading into the plate.

IV. Antibody Purification

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

A. Protocol: Antibody Purification Using Vacuum Filtration

1. Assemble the Capturem Protein A 24-Well Plate – HC (“Capturem plate” thereafter) in a filtration device together with a 24-well collection plate (supplied by the user—see Section II.B).
2. Add 2 ml of 1X Capturem Protein A Buffer, or your chosen buffer from Section II.B.1, to each well of the Capturem plate 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 plate in the 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 = 14.504×10^{-3} psi = 0.987×10^{-3} atm

3. Load 0.5–4.3 ml of diluted antibody (from Section III) into each well of the equilibrated Capturem 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 antibody analysis. Reassemble the Capturem plate in the filtration device together with a new collection plate. Add 4 ml of Capturem Protein A Buffer to each well of the Capturem plate to wash the wells. 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 wash for antibody analysis.
4. Elute your antibody from the Capturem plate as follows:
   a. Add 150–250 µl of 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 filtration device for the first elution.
   b. Add 1.5 ml of Elution Buffer to each well of the Capturem 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 now contain your eluted antibodies.

NOTES:

- If you use glycine as the Elution Buffer, only 1/10 the volume of the Neutralization Buffer needs to be added. If using 0.1 M citric acid, make sure to adjust the pH to an appropriate level for your sample; we found that for 1.5 ml of 0.1 M citric acid, 250 µl of Neutralization Buffer is required to neutralize.
- Most of the antibody is recovered in one elution of 1.5 ml elution buffer. If you do not recover your antibody in one elution, please, repeat Step 5 and perform a second elution.

5. Measure the amount of antibody in both eluates using UV 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 24-Well Plate - HC (“Capturem plate” thereafter) securely on top of a 24-well collection plate (supplied by the user—see Section II.B).
2. Add 2 ml of 1X Capturem Protein A Buffer, or your chosen buffer from Section II.B.1, to each well of the Capturem plate to equilibrate the wells. Centrifuge at 2,000*g* for 4 min at room temperature. Make sure wells are drained, remove the flowthrough and discard it along with the collection plate. Place the Capturem plate securely on top of a new 24-well collection plate.

NOTES:

- If wells did not drain fully, centrifuge again at 2,000g for 4 min.
- 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 or $g = (1.12) \times (R) \times (rpm/1{,}000)^2$
  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.

3. Load 0.5–4.3 ml of diluted antibody (from Section III) into each well of the equilibrated Capturem plate. Centrifuge at 2,000*g* for 4 min at room temperature. Save the collection plate containing the flowthrough for antibody analysis. Place the Capturem plate securely on top of a new 24-well collection plate.
4. Add 4 ml of 1X Capturem Protein A Buffer to each well of the Capturem plate to wash. Centrifuge at 2,000*g* for 4 min at room temperature. Save the collection plate containing the wash for antibody analysis.
5. Elute your antibody from the Capturem plate as follows:
   a. Add 150–250 µl of 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 1.5 ml of Elution Buffer to each well of the Capturem plate. Centrifuge at 2,000*g* for 4 min at room temperature.

NOTES:

- If you use glycine as the Elution Buffer, only 1/10 the volume of the Neutralization Buffer needs to be added. If using 0.1 M citric acid, make sure to adjust the pH to an appropriate level for your sample; we found that for 1.5 ml of 0.1 M citric acid, 250 µl of Neutralization Buffer is required to neutralize.
- Most of the antibody is recovered in one elution of 1.5 ml elution buffer. If you do not recover your antibody in one elution, please, repeat Step 5 and perform a second elution

6. Measure the amount of antibody in the eluate using UV 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.

C. Protocol: Antibody Purification Using Positive Pressure

1. Place the Capturem Protein A 24-Well Plate - HC ("Capturem plate" thereafter) securely on top of a 24-well collection plate (supplied by the user—see Section II.B).
2. Add 2 ml of 1X Capturem Protein A Buffer, or your chosen buffer from Section II.B.1, to each well of the Capturem plate to equilibrate the wells. Apply positive pressure of 10 psi at room temperature until all liquid passed through the wells. Make sure wells are drained completely, remove the flowthrough and discard it along with the collection plate. Place the Capturem plate securely on top of a new 24-well collection plate.

NOTE: If wells did not drain fully, apply positive pressure again at 10 psi.

3. Load 0.5–4.3 ml of diluted antibody (from Section III) into each well of the equilibrated Capturem plate and apply 10 psi at room temperature. Save the collection plate containing the flowthrough for antibody analysis. Place the Capturem plate securely on top of a new 24-well collection plate.
4. Add 4 ml of Capturem Protein A Buffer to each well of the Capturem plate and apply 10 psi at room temperature to wash. Save the collection plate containing the wash for antibody analysis.

5. Elute your antibody from the Capturem plate as follows:

   a. Add 150 µl of 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 1.5 ml of Elution Buffer to each well of the Capturem plate and apply 10 psi at room temperature.

NOTES:

- If you use glycine as elution buffer, only 1/10 of the neutralization buffer needs to be added. If using 0.1 M citric acid, please make sure to adjust the pH; we found that for 1.5 ml of 0.1 M citric acid, 250 µl of Neutralization Buffer is required to neutralize.
- Most of the antibody is recovered in one elution of 1.5 ml elution buffer. If you do not recover your antibody in one elution, please, repeat Step 5 and perform a second elution

6. Measure the amount of antibody in the eluate using UV absorbance at 280 nm or another colorimetric protein analysis method. One OD_{280} unit typically corresponds to 0.73 mg/ml of IgG.

Appendix A. Troubleshooting Guide

Table 1. Troubleshooting guide

Problem	Possible explanation	Solution
Background bands/poor purity	Nonspecific binding of proteins to membrane	Add an additional wash step with Capturem Protein A Buffer or Wash Buffer.
Minimal recovery	The sample contains more antibody than the Protein A 24-Well Plate - HC has capacity for.	Reduce the amount of sample added to each well. If you need to purify more antibody, consider splitting your sample into two separate wells or use Capturem Protein A Maxi Columns which have a higher capacity (1 mg).
Low yield of purified antibody	Poor binding affinity of the IgG subtype used. Different subtype classes and species bind differently to Protein A. Please, refer to Roque, A. C. A., Silva, C. S. O. & Taipa, M. A. Affinity-based methodologies and ligands for antibody purification: advances and perspectives. *J. Chromatogr. A* **1160,** 44–55 (2007).	Verify that Protein A-based purification is suitable for the IgG subtype class and species used. Alternatively, consider using Protein G-based purification instead.
Low yield of purified antibody	pH of the binding buffer was altered by the hybridoma, serum or cell culture medium used.	Check and make sure the pH of the chosen binding buffer is in the optimal range for your IgG sample.
Low yield of purified antibody	Suboptimal dilution	Make sure to use the optimal dilution of your sample. For hybridoma medium, 1:2 to 1:4 dilutions were found to work well, but it is sample dependent.
Antibody does not elute	Elution conditions too mild	• Follow the instructions and use 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.
Well does not fully drain	Clogging due to particles or very viscous sample	• Preclarify the solution by centrifugation or using a 0.5-micron filter. • Repeat spin at 2,000*g* for 4 min. • Reapply positive pressure at 10 psi.

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