I. Introduction

This protocol is provided for **Capturem Protein A Miniprep Columns** (Cat. No. 635717), single-use disposable columns for simple, rapid purification of antibodies from animal sera, ascites fluid, cell culture media, and other sources. Each column can hold up to 850 µl 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

- 12 Capturem Protein A Miniprep Columns (Cat. No. 635717)

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. However, 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 Tubes

Each purification will require three additional standard 2-ml collection tubes, with or without a cap. These tubes should be used throughout the protocol to collect flowthrough samples that will be saved for SDS-PAGE analysis and/or colorimetric protein assays (e.g., Bradford assays).

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

1. **Equilibrate:** Add 800 µl Equilibration Buffer (we recommend Protein A IgG Binding Buffer from Thermo Fisher Scientific) to a spin column which has been placed in the provided collection tube, in order to equilibrate the column. Centrifuge at 1,000*g* for 1 min at room temperature. Remove the flowthrough and discard it along with the collection tube, then place the column in a new collection tube (supplied by the user—see Section II.B).
2. **Bind:** Load 100–800 µl diluted sample (see Section III) onto the equilibrated spin column. Centrifuge at 1,000*g* for 1 min at room temperature. Save the collection tube containing the sample flowthrough for protein analysis and transfer the spin column to a new collection tube (supplied by the user).

   NOTE: For proteins expressed at low levels, the flowthrough or additional filtered lysate (up to 800 µl) may be reloaded onto the column. However, we do not recommend reloading more than two times.
3. **Wash:** Add 800 µl Wash Buffer to the spin column. Centrifuge at 1,000*g* for 1 min at room temperature. Save the collection tube containing the wash flowthrough for protein analysis and transfer the spin column to a new collection tube (supplied by the user).
4. **Elute:** Before inserting the spin column into a new collection tube for elution, add 10–30 µl Neutralization Buffer (1/10 the volume of Elution Buffer to be used) to the collection tube. Then insert the column into the collection tube and add 100–300 µl Elution Buffer to the column. Centrifuge at 1,000*g* for 1 min at room temperature and vortex the contents of the collection tube to mix your eluted antibody with the Neutralization Buffer in the tube. The eluted antibody is now ready for analysis.

   NOTE: ≥80–90% of your antibody can be eluted with 100 µl of Elution Buffer.
5. Measure the amount of antibody in your flowthrough samples from Steps 2 and 3, and your eluate from Step 4, 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 3 of Section IV.
Low percentage recovery	The sample contains more antibody than the Protein A Maxi Column has the capacity to bind.	Reduce the amount of sample added. If you need to purify more antibody, consider using Capturem Protein A 24 Plate or Maxi kit which have a higher capacities.
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 4 of Section IV.
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 1,000*g* for 1 min.

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