I. Introduction

This protocol is provided for **Capturem Protein G Maxiprep Columns** (Cat. No. 635727), single-use disposable columns for simple, rapid purification of antibodies from animal sera, ascites fluid, cell culture media, and other sources. Each well can hold up to 23 ml of sample and requires a minimum elution volume of 1 ml. Depending upon the sample type, species, and antibody isotype the binding capacity varies. More information about bed volume and capacities can be found on our website.

II. Materials and Reagents

A. Components

- 6 Capturem Protein G Maxiprep Columns (Cat. No. 635727)

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. We highly **recommend using a binding buffer with an optimal pH of 5,** preferably Protein G IgG binding buffer from Thermo Fisher Scientific, Cat. No. 21019. 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 G IgG binding buffer. You can also use 20–100 mM sodium acetate containing 0.15–2 M NaCl, pH 5.0, or 100–500 mM sodium phosphate containing 0.15–2 M NaCl, pH 7.0.
- **Wash Buffer 2:** Dulbecco`s PBS Buffer w/o Ca^{2+}, Mg^{2+} (pH 7.5)
- **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 four additional standard 50-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). 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 tubes are required.

III. Sample Preparation

1. Follow the standard protocols for preparing antibody samples. We recommend diluting the antibody sample with binding buffer in the range of 1:2–1:15 sample to binding buffer. A 1:2 – 1:4 dilution is recommended for hybridoma samples. We suggest a dilution of 1:15 for serum samples, which may differ depending on the species and may require further optimization to obtain high yields and purity.
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. Add 6 ml of Equilibration/Binding/Wash Buffer, preferably Protein G 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 2,000*g* for 2 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. Load 2–20 ml of diluted sample (see Section III) onto the equilibrated spin column. Centrifuge at 2,000*g* for 2–4 min at room temperature. Save the collection tube containing the sample flowthrough for antibody analysis and transfer the spin column to a new collection tube (supplied by the user).

   NOTE: For antibodies present at low concentrations, the flowthrough or additional filtered sample (up to 25 ml) may be reloaded onto the column. However, we do not recommend reloading more than once.
3. Add 6 ml of Equilibration/Binding/Wash Buffer to the spin column. Centrifuge at 2,000*g* for 2 min at room temperature. Save the collection tube containing the wash flowthrough for antibody analysis and transfer the spin column to a new collection tube (supplied by the user).
4. Wash a second time by adding 6 ml of **Wash Buffer 2** (Dulbecco's PBS Buffer w/o Ca^{2+}, Mg^{2+}, pH 7.5) to the spin column. Centrifuge at 2,000*g* for 2 min. at room temperature. Save the collection tube containing the flowthrough for antibody analysis and transfer the spin column to a new collection tube (supplied by the user).

   NOTE: If significant background due to non-specific binding of other proteins is observed, please, include after step 5 an additional wash step by adding 2mL Wash Buffer 2 (PBS w/o Ca, Mg). Centrifuge at 2,000*g* for 2 min. at room temperature to drain the wells into the collection plate. Save the collection plate containing the flowthrough for antibody analysis.
5. Before inserting the spin column into a new collection tube for elution, add 50–150 µl of 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 0.5–1.5 ml of Elution Buffer to the column. Centrifuge at 2,000*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.
6. Remove the spin column from the collection tube and insert it into a new 50-ml collection tube containing Neutralization Buffer (1/10 of the volume of Elution Buffer to be used). Perform a second elution as described in Step 5, making sure to mix your eluted antibody with the Neutralization Buffer in the tube.
7. The eluted antibody is now ready for analysis. 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.

   NOTE: Most of the antibody is recovered in the two elution steps.

Appendix A. Troubleshooting Guide

Table 1. Troubleshooting Guide

Problem	Possible Explanation	Solution
Background bands/poor purity	Non-specific binding of proteins to membrane	Add an additional wash step with Wash Buffer 2 (PBS) or TBS
Minimal recovery	The sample contains more antibody than the Protein G Maxiprep has capacity for.	Reduce the amount of sample added.
Low yield of purified antibody	Poor binding affinity of 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 G based purification is suitable for the IgG subtype class and species used. Alternatively, consider using Protein A based purification instead.
Low yield of purified antibody	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 5). We recommend Protein G IgG buffer from ThermoFisher Scientific as binding buffer.
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 were found to work well, but it is sample dependent.
Antibody does not elute	Elution conditions too mild	• Follow the instructions 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 different buffer than the one recommended.
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 spin at 2,000*g*

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