I. Introduction

This protocol is provided for the **Capturem Protein G 24-Well Plate** (Cat. No. 635732), 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 ml of sample and requires a minimum elution volume of 500 µl. 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

- 1 Capturem Protein G 24-Well Plate (Cat. No. 635732)

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. Alternately, 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. Alternatively, Pierce Protein G IgG Binding Buffer (Thermo Fisher Scientific, Cat. # 21019) may be used.
- **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 Plates

- **24-well collection plates:** Each purification will require four standard 24-well collection plates (e.g., E&K Scientific, Cat. No. EK-2239, 24-well storage plate, square top 10 ml, deep well, round bottom). 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 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 G 24-Well Plate 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 G 24-Well Plate is compatible with standard 96-well plate centrifuges (e.g., Eppendorf Centrifuge 5804 R with Deepwell-plate rotor, A-2-DWP).
- **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 binding buffer. We recommend 1:2 – 1:4 dilution with binding buffer for hybridoma samples; and 1:15 dilution with binding buffer 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.
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) or centrifugation in a standard 96-well plate centrifuge (Protocol B).

A. Protocol: Antibody Purification Using Vacuum Filtration

1. Assemble the Capturem Protein G 24-Well Plate in a filtration device together with a 24-well collection plate (supplied by the user—see Section II.B).
2. Add 2 ml of Equilibration/Binding/Wash Buffer to each well of the Capturem Protein G 24-Well Plate to equilibrate the wells. We recommend Protein G IgG binding buffer from ThermoFisher Scientific. 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 G 24-Well 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 x 10^{-3} psi = 0.987 x 10^{-3} atm
3. Load 0.5–4.5 ml of diluted antibody (from Section III) into each well of the equilibrated Capturem Protein G 24-Well Plate. We recommend Protein G IgG binding buffer from ThermoFisher Scientific. 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 Protein G 24-Well Plate in the filtration device together with a new collection plate.
4. Add 2 ml of Wash Buffer to each well of the Capturem Protein G 24-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 antibody analysis.

5. Wash a second time by adding 2 ml of **Wash Buffer 2** (PBS Buffer w/o Ca^{2+}, Mg^{2+}, pH 7.5) to each well of the Capturem Protein G 24-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 antibody analysis.

   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). 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.

6. Elute your antibody from the Capturem Protein G 24-Well Plate as follows:

   a. Add 50–150 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 0.5–1.5 ml of Elution Buffer to each well of the Capturem Protein G 24-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 now contain your eluted antibodies.

   c. Perform a second elution by repeating Steps a and b above using a new collection plate. The wells of the collection plates should contain your eluted antibodies, which are now ready for analysis.

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

7. 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 G 24-Well Plate securely on top of a 24-well collection plate (supplied by the user—see Section II.B).

2. Add 2 ml of Equilibration Buffer, preferably Protein G IgG binding buffer from Thermo Fisher Scientific, to each well of the Capturem Protein G 24-Well Plate to equilibrate the wells. Centrifuge at 600*g* for 2 min at room temperature. Remove the flowthrough and discard it along with the collection plate. Place the Capturem Protein G 24-Well Plate securely on top of a new 24-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: $RCF \text{ or } g = (1.12) \times (R) \times (rpm/1{,}000)^2$

   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.

   If a well does not drain entirely, centrifuge again at 600*g* for 2 min.

3. Load 0.5–4.5 ml of diluted antibody (from Section III) into each well of the equilibrated Capturem Protein G 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for antibody analysis. Place the Capturem Protein G 24-Well Plate securely on top of a new 24-well collection plate.
4. Add 2 ml of Wash Buffer to each well of the Capturem Protein G 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for antibody analysis.
5. Wash a second time by adding 2 ml of **Wash Buffer 2** (PBS Buffer w/o Ca^{2+}, Mg^{2+}, pH 7.5) to each well of the Capturem Protein G 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for antibody analysis.

   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 600*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.
6. Elute your antibody from the Capturem Protein G 24-Well Plate as follows:
   a. Add 50–150 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 for the first elution.
   b. Add 0.5–1.5 ml of Elution Buffer to each well of the Capturem Protein G 24-Well Plate. Centrifuge at 600*g* for 2 min at room temperature. The wells of the collection plate now contain your eluted antibodies.
   c. Perform a second elution by repeating Steps a and b above using a new collection plate. The wells of the collection plates should contain your eluted antibodies, which are now ready for analysis.

   NOTE: Most of the antibody is recovered in two elution steps.
7. 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.

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 24-Well Plate 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 24 wells or use Capturem Protein G Maxi kit which has a higher capacity (1-2mg).
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 600g

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