I. Introduction

This protocol is provided for the **Capturem™ Streptavidin 96-Well Plate** (Cat. No. 635734), single-use disposable 96-well plate. The protocol is designed for the enrichment of target proteins (e.g., antibodies) by biotinylated antibodies immobilized on the streptavidin membrane without incubation. Each well can hold up to 1 ml of sample and requires a minimum elution volume of 50 µl. The amount of target antibody that can be captured by a biotinylated capture antibody depends on the quality of the antibodies used.

II. Materials and Reagents

A. Components

- 1 Capturem Streptavidin 96-Well Plate

B. Additional Materials Required

1. Enrichment Buffers

This plate is compatible with all standard buffers typically used for antibody research, such as phosphate- and glycine-based buffers. Several standard buffer options are listed below, but others can also be used as needed.

- **Equilibration/Binding/Wash Buffer:** We recommend using Pierce Protein G IgG Binding Buffer (Thermo Fisher Scientific, Cat. No. 21019) for the binding step. Alternatively, other buffers like Pierce Protein A IgG Binding Buffer (Thermo Fisher Scientific, Cat. No. 21001) or 50–100 mM sodium phosphate containing 1 mM EDTA and 1 M NaCl, pH 7.0 may be used; however, they may show lower binding.

  NOTE: We do not recommend low-salt PBS buffer for the binding step.

- **Wash Buffer 2**: PBS (without Mg^{2+}, Ca^{2+}), pH 7.5
- **Elution Buffer:** 0.1 M glycine, pH 2.5
- **Neutralization Buffer:** 1 M Tris, pH 8.5

2. Consumables

- **96-well collection plates:** Each enrichment will require seven 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. Alternatively, if only the eluates are to be analyzed, the flowthrough from the equilibration and wash steps can be discarded.
- **96-well plate filtration device:** The Capturem Streptavidin 96-Well Plate is compatible with standard 96-well plate filtration devices, such as the NucleoVac 96 Vacuum Manifold (Cat. No. 740681).
- **96-well positive pressure device:** The Capturem Streptavidin 96-Well Plate is compatible with standard 96-well plate positive pressure devices, such as the Waters Positive Pressure-96 Processor Manifold (Cat. No. 186006961).
- **96-well plate centrifuge:** The Capturem Streptavidin 96-Well Plate is compatible with standard 96-well plate centrifuges (e.g., the Eppendorf Centrifuge 5804 R with Deepwell-plate rotor, A-2-DWP).
- Multichannel pipette

III. Sample Preparation

1. Follow standard procedures for preparing antibody samples. We recommend diluting the antibody sample in the range of 1:1 (sample:binding buffer). We recommend a 1:1 dilution with binding buffer for hybridoma samples.
2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto the wells.

IV. Antibody Enrichment Protocols

Enrichment of target proteins or 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. Target Protein or Antibody Enrichment Using Vacuum Filtration

1. Assemble the Capturem Streptavidin 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).

   NOTE: All the collection plates referenced from this point on are supplied by the user (see Section II.B).

   a. Add 800 µl of Equilibration Buffer to each well of the streptavidin plate using a multichannel 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 Streptavidin 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. Load 200–1,000 µl of biotinylated antibody (from Section III) onto each well of the equilibrated streptavidin 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 streptavidin plate in the 96-well plate filtration device together with a new collection plate.
4. Add 400 µl of Wash Buffer to each well of the streptavidin plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the streptavidin plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.
5. Load 200–1,000 µl of sample containing the diluted target protein or antibody (from Section III) onto each well of the streptavidin plate. Use a vacuum (~0.3–0.4 bar) to run the samples through the Capturem plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis. Reassemble the streptavidin plate in the 96-well plate filtration device together with a new collection plate.
6. Add 400 µl of Wash Buffer to each well of the streptavidin plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the streptavidin plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.
7. Add 400 µl of Wash Buffer 2 (PBS) to each well of the streptavidin plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the streptavidin plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.

8. Elute your enriched target protein or antibody from the streptavidin plate as follows:
   a. Add 5–20 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 streptavidin plate in the 96-well plate filtration device for the first elution.
   b. Add 45–180 µl of Elution Buffer (9/10 of the total volume of Elution Buffer to be used) to each well of the streptavidin plate. Use a vacuum (~0.3–0.4 bar) to run the buffer through the streptavidin plate wells into the collection plate. The wells of the collection plate should contain your eluted antibodies.
   c. In a new collection plate, perform a second elution by repeating Steps 8a and 8b above.
   d. In a new collection plate, perform a third elution by repeating Steps 8a and 8b above.
   e. The eluted antibodies are now ready for downstream analysis.

   NOTE: We have observed that most of the antibodies are recovered in 2–3 elution steps.
9. Measure the amount of protein or antibody in eluates using UV absorbance at 280 nm or another colorimetric protein analysis method. One OD280 unit typically corresponds to 0.73 mg/ml of IgG. The purity of the eluted proteins or antibodies can be analyzed by SDS-PAGE, size exclusion chromatography, etc.

B. Target Protein or Antibody Enrichment Using Centrifugation

1. Place the Capturem Streptavidin 96-Well Plate securely on top of a 96-well collection plate.

   NOTE: All the collection plates referenced from this point on are supplied by the user (see Section II.B).
2. Add 800 µl of Equilibration Buffer to each well of the streptavidin plate using a multichannel pipette, to equilibrate the wells. Centrifuge at 500*g* for 2 min at room temperature. Remove the flowthrough and discard it along with the collection plate. Place the Capturem Streptavidin 96-Well Plate securely on top of a new 96-well collection plate.

   NOTE: The following formula can be used to convert RPM to RCF or G-force, if not available on the centrifuge:

   $$\textbf{RCF or G-force} = 1.12 \times R \times (RPM/1{,}000)^2$$

   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 200–1,000 µl of biotinylated antibody onto each well of the equilibrated streptavidin plate. Centrifuge at 500*g* for 2 min. Save the collection plate containing the flowthrough for protein analysis. Reassemble the streptavidin plate in the 96-well plate filtration device together with a new collection plate.
4. Add 400 µl of Wash Buffer to each well of the streptavidin plate. Centrifuge at 500*g* for 2 min. Save the collection plate containing the flowthrough for protein analysis.
5. Load 200–1,000 µl of sample containing the diluted target protein or antibody (from Section III) onto each well of the equilibrated streptavidin plate. Centrifuge at 500*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for protein analysis. Place the streptavidin plate securely on top of a new 96-well collection plate.
6. Add 400 µl of Wash Buffer to each well of the streptavidin plate. Centrifuge at 500*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for protein analysis.
7. Add 400 µl of Wash Buffer 2 (PBS) to each well of the streptavidin plate. Centrifuge at 500*g* for 2 min at room temperature. Save the collection plate containing the flowthrough for protein analysis.

8. Elute your antibody from the Capturem Streptavidin 96-Well Plate as follows:
   a. Add 5–20 µl of Neutralization Buffer (1/10 the volume of Elution Buffer to be used) to each well of a new collection plate for the first elution.
   b. Add 45–180 µl of Elution Buffer (9/10 the total volume of Elution Buffer to be used) to each well of the streptavidin plate. Centrifuge at 500g for 2 min at room temperature. The wells of the collection plate should contain your eluted antibodies.
   c. In a new collection plate, perform a second elution by repeating Steps 6a and 6b above.
   d. In a new collection plate, perform a third elution by repeating Steps 6a and 6b above.
   e. The eluted antibodies are now ready for downstream analysis.

NOTE: We have observed that most of the antibody is recovered in 2–3 elution steps.

9. Measure the amount of protein or antibody in 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 proteins or antibodies can be analyzed by SDS-PAGE, size exclusion chromatography etc.

C. Target Protein or Antibody Enrichment Using Positive Pressure

1. Assemble the Capturem Streptavidin 96-Well Plate in a 96-well positive pressure-96 manifold together with a 96-well collection plate (supplied by the user—see Section II.B).

NOTE: All the collection plates referenced from this point on are supplied by the user (see Section II.B).

b. Add 800 µl of Equilibration Buffer to each well of the streptavidin plate using a multichannel pipette, to equilibrate the wells. Use a positive pressure (10 psi) 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 Streptavidin 96-well Plate in the 96-well positive pressure device together with a new collection plate.

NOTE: Make sure wells are drained completely. Use positive pressure again if wells are still wet.

3. Load 200–1,000 µl of biotinylated antibody (from Section III) onto each well of the equilibrated streptavidin plate. Use a positive pressure (10 psi) 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 streptavidin plate in the 96-well positive pressure device together with a new collection plate.
4. Add 400 µl of Wash Buffer to each well of the streptavidin plate. Use positive pressure (10 psi) to run the buffer through the streptavidin plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.
5. Load 200–1,000 µl of sample containing the diluted target protein or antibody (from Section III) onto each well of the streptavidin plate. Use positive pressure (10 psi) to run the samples through the Capturem plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis. Reassemble the streptavidin plate in the 96-well plate positive pressure device together with a new collection plate.
6. Add 400 µl of Wash Buffer to each well of the streptavidin plate. Use positive pressure (10 psi) to run the buffer through the streptavidin plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.
7. Add 400 µl of Wash Buffer 2 (PBS) to each well of the streptavidin plate. Use positive pressure (10 psi) to run the buffer through the streptavidin plate wells into the collection plate. Save the collection plate containing the flowthrough for protein analysis.

8. Elute your enriched target protein or antibody from the streptavidin plate as follows:
   a. Add 5–20 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 streptavidin plate in the 96-well plate positive pressure device for the first elution.
   b. Add 45–180 µl of Elution Buffer (9/10 the total volume of Elution Buffer to be used) to each well of the streptavidin plate. Use positive pressure (10 psi) to run the buffer through the streptavidin plate wells into the collection plate. The wells of the collection plate should contain your eluted antibodies.
   c. In a new collection plate, perform a second elution by repeating Steps 8a and 8b above.
   d. In a new collection plate, perform a third elution by repeating Steps 8a and 8b above.
   f. The eluted antibodies are now ready for downstream analysis.

   NOTE: We have observed that most of the antibody is recovered in 2–3 elution steps.

9. Measure the amount of protein or antibody in 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 proteins or antibodies can be analyzed by SDS-PAGE, size exclusion chromatography, etc.

V. Additional Capture Protocols

For other enrichment protocols (i.e., oligo capture and in-solution antibody capture), see the Capturem Streptavidin Miniprep User Manual. Documents for our products are available for download at **takarabio.com/manuals.** The volumes and buffers are the same for the 96-well plate and the mini spin columns, only the vacuum filtration or centrifugation steps are different.

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 (PBS) or TBS after loading the sample containing the target antibody or protein (Section IV.C, Steps 6–7).
Low yield of purified antibody	Poor binding affinity of the target antibody to the capture antibody	Instead of successive capture, pre-incubate the biotinylated capture antibody with the target antibody and then pass the mix diluted in binding buffer through the wells. Skip Steps 3 and 4.
	Suboptimal dilution	Make sure to use the optimal dilution of your sample. For hybridoma medium, dilutions of 1:2 to 1:4 were found to work well, but this is sample dependent.
Target 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 recommended one.
Wells do not fully drain	Clogging due to particles or a very viscous sample	• Pre-clarify the solution by centrifugation or using a 0.8-micron filter. • Repeat centrifugation at a higher speed, e.g., 1,000*g* for 2 min.

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