I. Introduction

This protocol is provided for **Capturem Streptavidin Minipreps** (Cat. No. 635733), containing single-use disposable columns. The protocol is designed for the enrichment of target proteins (e.g., antibodies) by incubation of a sample solution with biotinylated antibodies and subsequent capture of the antibody-target complex. Each column can hold up to 850 µl of sample and requires a minimum elution volume of 100 µl. The amount of target antibody that can be captured by a biotinylated capture antibody depends on the quality of both antibodies used.

II. Materials and Reagents

A. Components

- 20 Capturem Streptavidin Miniprep Columns

B. Additional Materials Required

Each enrichment/capture will require additional standard 2-ml collection tubes, with or without a cap. These tubes should be used throughout the protocol to collect flowthrough samples to be saved for SDS-PAGE analysis and/or colorimetric protein assays (e.g., Bradford assays). In addition to collection tubes, each type of capture experiment will need a set of buffers detailed below:

1. Oligo Capture

- **Equilibration/Binding: 5 mM** Tris, 0.5 mM EDTA, 1 M NaCl, pH 7. Alternatively, a simple Tris-buffered saline (TBS) OR 10 mM Tris, 1 mM EDTA, 1 M NaCl can be used.

2. In-Solution Antibody Capture

- **Equilibration/Binding/Wash Buffer 1:** Pierce Protein G IgG Binding Buffer (Thermo Fisher Scientific, Cat. No. 21019) 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 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

3. Successive Capture

- **Equilibration/Binding/Wash Buffer 1:** Pierce Protein G IgG Binding Buffer (Thermo Fisher Scientific, Cat. No. 21019). 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 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

4. Capture and Boil
   - **Equilibration/Binding/Wash Buffer 1:** Pierce Protein G IgG binding buffer (ThermoFisher Scientific, Cat. No. 21019). Alternatively, other buffers like Pierce Protein A IgG Binding Buffer (Thermo Fisher Scientific, Cat. No. 21001) or a phosphate-based buffer (50–100 mM) can be used that contains 1 mM EDTA and 1M NaCl, pH 7 may be used; however, they may show lower binding.

     NOTE: We do not recommend low-salt PBS for the binding step.
   - **Wash buffer 2:** PBS (without Mg^{2+}, Ca^{2+}), pH 7.5
   - **Boiling Buffer:** SDS loading buffer (6X) diluted in Millipore water

     NOTE: It may be useful to include a blocking agent (e.g., 100 µg BSA) in the first wash step.

III. Capture Protocols

NOTES:

- 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.
- Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto the plate/column.

A. Oligo Capture Sample Preparation and Capture Protocol

1. Follow standard procedures for preparing oligo samples.
2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto a streptavidin spin column.
3. Add 800 µl Equilibration Buffer to a spin column which has been placed in the provided collection tube, to equilibrate the column. Centrifuge at 500*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).
4. Load 50–800 µl diluted oligo sample onto the equilibrated spin column. Centrifuge at 500*g* for 1 min at room temperature. You can save the collection tube containing the sample flowthrough for analysis.

   NOTE: Depending on the buffer, 500*g* might not be sufficient to pass all of the solution through the membrane. However, we do not recommend reloading the flowthrough onto the column. Simply increase the time or the RCF to pass the remaining solution.
5. The immobilized oligos can now be used for downstream applications such as ribosomal RNA removal or target enrichment.

B. In-Solution Ab Capture Sample Preparation and Capture Protocol

1. Follow the standard protocols for preparing antibody samples. We recommend diluting the antibody mixture (biotinylated primary antibody and target antibody) in the range of 1:1 (sample :binding buffer).

   NOTE: Alternatively, the target antibody can be captured successively (see Successive Capture protocol, Section III.C).
2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto a streptavidin spin column.

3. Add 800 µl Equilibration Buffer to a spin column which has been placed in the provided collection tube, to equilibrate the column. Centrifuge at 500*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.

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

4. Load 100–800 µl diluted sample onto the equilibrated spin column. Centrifuge at 500*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.

   NOTE: Depending on the buffer, 500*g* might not be sufficient to pass all of the solution through the membrane. However, we do not recommend reloading the flowthrough onto the column. Simply increase the time or the RCF to pass the remaining solution.

5. Add 400 µl Wash Buffer to the spin column. Centrifuge at 500*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.
6. Remove the spin column and place in a new 2-ml collection tube to perform a second wash using Wash Buffer 2 (PBS) as per Step 5.
7. Before inserting the spin column into a new collection tube for elution, add 10µl Neutralization Buffer (1/10 of the volume of Elution Buffer to be used) to the collection tube. Then insert the column into the collection tube and add 90 µl Elution Buffer to the column. Centrifuge at 500*g* for 1 min at room temperature. Remove the spin column and vortex the contents of the collection tube to mix your eluted antibody with the Neutralization Buffer in the tube.
8. Place the spin column in a new 2-ml collection tube containing neutralization buffer (1/10 of the volume of Elution Buffer to be used) to perform a second elution as per Step 7. Remove the spin column and vortex the tube to mix the eluate and Neutralization Buffer.
9. Place the spin column in a new 2-ml collection tube containing neutralization buffer (1/10 of the volume of Elution Buffer to be used) to perform a third elution as per Step 7. Remove the spin column and vortex the tube to mix the eluate and Neutralization Buffer.
10. The eluted target antibody is now ready for analysis. Measure the amount of antibody in all 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 antibody can be analyzed by SDS-PAGE, size exclusion chromatography, etc.

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

C. Successive Capture

1. Follow the standard protocols for preparing antibody samples. We recommend diluting the antibodies in the range of 1:1 sample to binding buffer.
2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto a spin column.
3. Add 800 µl Equilibration Buffer to a spin column which has been placed in the provided collection tube, to equilibrate the column. Centrifuge at 500*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.

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

4. Load 100–800 µl diluted biotinylated antibody onto the equilibrated spin column. Centrifuge at 500*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.

   NOTE: Depending on the buffer, 500*g* might not be sufficient to pass all of the solution through the membrane. However, we do not recommend reloading the flowthrough onto the column. Simply increase the time or the RCF to pass the remaining solution.

5. Add 400 µl Wash Buffer 1 to the spin column. Centrifuge at 500*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.
6. Load 100–800 µl diluted sample (secondary antibody or antigen) onto the equilibrated spin column. Centrifuge at 500*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.
7. Add 400 µl Wash Buffer 1 to the spin column. Centrifuge at 500*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.
8. Remove the spin column and place in a new 2-ml collection tube to perform a second wash with Wash Buffer 2 (PBS) as per Step 7.
9. Before inserting the spin column into a new collection tube for elution, add 10 µl Neutralization Buffer (1/10 of the volume of Elution Buffer to be used) to the collection tube. Then insert the column into the collection tube and add 90 µl Elution Buffer to the column. Centrifuge at 500*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.
10. Place the spin column in a new 2-ml collection tube containing neutralization buffer (1/10 of the volume of Elution Buffer to be used) to perform a second elution as per Step 9. Remove the spin column and vortex the tube for mixing.
11. Place the spin column in a new 2-ml collection tube containing neutralization buffer (1/10 of the volume of Elution Buffer to be used) to perform a third elution as per Step 9. Remove the spin column and vortex the tube for mixing.
12. The eluted antibody is now ready for analysis. Measure the amount of secondary antibody in all 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 antibody can be analyzed by SDS-PAGE, size exclusion chromatography, etc.

D. Capture and Boil

1. We recommend diluting the sample in the range of 1:1 (sample:binding buffer).
2. Samples with visible precipitates must be clarified by centrifugation or filtered through a 0.8-micron filter before loading onto a spin column.
3. Add 800 µl Equilibration Buffer to a spin column which has been placed in the provided collection tube, to equilibrate the column. Centrifuge at 500*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.

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

4. Load 100–800 µl diluted sample onto the equilibrated spin column. Centrifuge at 500*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: Depending on the buffer, 500*g* might not be sufficient to pass all of the solution through the membrane. However, we do not recommend reloading the flowthrough onto the column. Simply increase the time or the RCF to pass the remaining solution.

5. Add 400 µl Wash Buffer 1 to the spin column. Centrifuge at 500*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.
6. Remove the spin column and place in a new 2-ml collection tube to perform a second wash with Wash Buffer 2 (PBS) as per Step 5.
7. Insert the column into the collection tube, add 50 µl loading buffer to the column, and heat it up in a heating block (100°C) for 10 min. Centrifuge at 500*g* for 1 min at room temperature.
8. Repeat Step 5: Add 50 µl loading buffer to the column and heat it up in a heating block (100°C) for 10 min. Centrifuge at 1,000*g* for 1 min at room temperature.
9. The eluted protein is now ready for SDS Page analysis.

Appendix A. Troubleshooting Guide

Table 1. Troubleshooting Guide

Problem	Possible explanation	Solution
Nonspecific binding of target to membrane	Protein sticks to membrane	• Add blocking agent such as BSA to wash step • Wash with 0.1 M Tris buffer instead of PBS • Add an additional wash step
Low yield of target protein	Poor binding affinity of capture antibody to target or degraded antibody	Verify that the capture antibody binds well to the target, try a different capture antibody, or purchase fresh antibody

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