UBPBio
12635 E. Montview Blvd. Ste 100
Aurora, CO 80045, USA
Fax: 1-800-682-6801
Email: info@ubpbio.com
www.ubpbio.com

User's Guide
Streptavidin Magnetic Polymer Resin

I. Description

Streptavidin magnetic resins contain recombinant full-length streptavidin protein covalently coupled on non-porous magnetic polymer resins. The resin can be used for purification of biotinylated proteins or nucleic acids in cell culture or tissue extracts, studying protein-protein interactions, isolation of cell surface biotinylated cells, etc.

Comparing to agarose-based resins, non-porous magnetic polymer resins have much less non-specific binding, and suitable for rapid isolation and elution using a magnetic stand.

II. Streptavidin Magnetic Polymer Resin Specifications:

Resin: Non-porous superparamagnetic polymer
Ligand: Recombinant full-length streptavidin
Binding Capacity: ~ 3 nmol D-biotin per mg resin or 100 µL supplied resin (total rein volumes including buffer)
Resin Concentration: 10 mg/ml
Storage Buffer: 1X PBS, 0.01% Tween-20, 0.02% Sodium azide (NaN_3)
Storage Temperature: 4 ^{0}C

III. Important Notes:

1. Do not centrifuge, dry or freeze the magnetic resins, otherwise would cause resin aggregation.
2. Include 0.01 - 0.05% non-ionic detergent (e.g., Tween-20) in the binding buffer to prevent resin aggregation.
3. Low pH elution may be used for single-use applications. Maximal time for low pH elution is 10 minutes. Resins can be cleaned and reused after low pH elution.
4. Streptavidin resins can not be reused if elution conditions with boiling are used.

IV. Biotinylated Antibody and Protein Purification Protocols

Important Note: Conditions should be optimized by users for each application.

A. Biotinylated Antibody Purification Protocol

Additional Materials Required:

- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline containing 0.02% Tween-20, or 1X PBS containing 0.02% Tween-20
- Elution Buffer: 0.1M glycine, pH 2.0
- Neutralization Buffer: 1M Tris, pH 8.5
- Magnetic stand

Note: Mix resins thoroughly before each use by repeated inversion or gentle vortexing. Protease inhibitors may be added in the sample to prevent degradation of antigens and antibodies.

1. Pipette 100 µL (1.0 mg) of Streptavidin Magnetic Polymer Resins into a 1.5mL microcentrifuge tube. Add 500 µL of Binding/Wash Buffer to the resins and gently vortex to mix.

Note: 1.0 mg resin can be used for purification of ~ 0.2 - 0.4 mg biotinylated antibody.

2. Place the tube into a magnetic stand to collect resins against the wall of the microcentrifuge tube. Remove and discard the supernatant.
3. Add 1 mL Binding/Wash Buffer to the tube. Invert the tube several times to mix. Collect resins with a magnetic stand, then remove and discard the supernatant.

Note: make sure cleaned resins are not dried. You can add a little Binding/Wash buffer in the tube if you have to wait for the sample to be ready.

4. Add 500 µL antibody-containing sample to pre-washed magnetic resins and invert to mix well.
Note: Sample concentration should be optimized. If the sample volume is < 500 µL, dilute to 500 µL with Binding/Wash Buffer.
5. Rotate the tube to mix the sample and resins under room temperature for 1 hour, or under 4 ^{0}C for 4 -6 hours or overnight.
6. Collect resins with a magnetic stand, then remove and discard the supernatant.
Note: you may save the supernatant at Step 6 for characterization such as by SDS-PAGE.
7. Add 500 µL of Binding/Wash Buffer to the tube, rotate the tube for 5 min, collect resins with a magnetic stand, and then remove and discard the supernatant. Repeat the wash step twice.
Note: You may add 0.3 - 0.5M NaCl in the Binding/Wash Buffer in the wash steps to reduce non-specific binding.
8. Add 100 µL of Elution Buffer to the tube, mix well and incubate 10 minutes at room temperature with occasional mixing.
9. Collect resins with a magnetic stand, remove and save the supernatant that contains the eluted antibody.
10. Neutralize eluted antibody immediately by adding 15 µL Neutralization Buffer for each 100 µL of eluate.

C. Biotinylated Protein or protein Complex Purification Protocol
Additional Materials Required:
- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline containing 0.02% Tween-20, or 1X PBS containing 0.02% Tween-20
- Elution Buffer: 6M ultra-pure Urea in TBS or PBS, or 1X SDS reducing sample buffer
- Magnetic stand

Note: Mix resins thoroughly before each use by repeated inversion or gentle vortexing. Protease inhibitors may be added in the sample to prevent degradation of antigens and antibodies.

1. Pipette 50 µL (0.5mg) of Streptavidin Magnetic Polymer Resins into a 1.5mL microcentrifuge tube. Add 500 µL Binding/Wash Buffer to resins and gently vortex to mix.
2. Place the tube into a magnetic stand to collect resins against the wall of the microcentrifuge tube. Remove and discard the supernatant.
3. Add 1 mL Binding/Wash Buffer to the tube. Invert the tube several times to mix. Collect beads with a magnetic stand. Remove and discard the supernatant.
Note: make sure cleaned resins are not dried. You can add a little Binding/Wash buffer in the tube if you have to wait for the sample to be ready.
4. Add 500 µL sample to the pre-washed magnetic resins. Incubate at room temperature for 2-4 hours with mixing, or at 4 ^{0}C for 4-8 hours or overnight.
Note: Sample concentration should be optimized. If the sample volume is < 500 µL, dilute to 500 µL with Binding/Wash Buffer.
5. Collect resins with a magnetic stand, remove the supernatant and save for characterizations.
6. Add 500 µL Binding/Wash Buffer to the tube and gently mix for 5 min. Collect resins using a magnetic stand, remove and discard the supernatants. Repeat the wash step twice. You may save the supernatants in the wash steps for analysis.
Note: You may add 0.3 - 0.5M NaCl in the Binding/Wash Buffer in the wash steps to reduce non-specific binding. If you only need the biotinylated proteins, you can use a buffer containing 5 M urea to wash. However, if you want to detect protein complexes, use mild wash buffer conditions.
7. Add 100µL of Elution Buffer to the tube. Mix the tube and boil for 5-10 minutes. Collect resins using a magnetic stand. Collect and save the supernatant containing the target protein.
8. Alternatively, add 100 µL 1X SDS reducing sample buffer to the tube and boil samples for 5-10 minutes. Collect resins using a magnetic stand and save the supernatant containing the target antigen.
Note: The biotin and streptavidin interaction needs harsh elution conditions. Eluted proteins are denatured, can be used for mass spectrometric analysis and SDS-PAGE analysis. Urea is compatible for SDS-PAGE separation. Do not use guanidine hydrochloride to elute if you want to separate eluate on SDS-PAGE.

D. Troubleshooting

Problem	Cause	Solution
Low levels of protein/antibody were	Low biotinylated protein/antibody levels	Increase sample volume or concentrate

recovered	in the sample	the sample.
	Antibody and proteins were degraded	Add protease inhibitors in the sample.
	Not enough magnetic resins	Use more resins in the reaction.
	Antibody and protein did not elute	Increase elution boiling time, and/or use 6M urea in pH 2 to elute.
Non-specific proteins appear in eluate	Non-specific protein bounds on resins	Add NaCl to 0.3 - 0.5M and/or increase Tween-20 concentration to 0.05% in washing buffer. If you only need biotinylated proteins, use a wash buffer with 5 M urea.
Resin aggregation	Centrifuging, drying or freezing resins	Avoid centrifuging, drying or freezing resins.
	No non-ionic detergents in samples and in binding/wash buffer	add 0.02% - 0.05% Tween-20 in sample buffer, and binding/wash buffer.
Purified antibody showed bands at ~50 kDa appeared on the Western blot	Elution was performed under boiling when a rabbit primary or secondary antibody was used for Westernblotting	Perform antibody elution under room temperature.