# Protocol

TD-P Revision 3.0

**Creation Date: 5/25/2016**
**Revision Date: 12/17/2018**

## Ultra HBC™ Streptavidin Agarose Resin Protocols

## Introduction

GoldBio Ultra HBC™ Streptavidin agarose resin is a high biotin binding capacity resin used to purify biotinylated molecules in molecular biology. This resin is composed of 6% cross-linked agarose beads with covalently-bound streptavidin, a biotin-binding tetrameric 60 kD protein produced by the bacterium *Streptomyces avidinii* that can bind up to four moles of biotin per mole of protein with very high affinity. Streptavidin is often preferred because it is resistant to extreme pH, temperature, denaturants, detergents, and proteolytic enzymes. In addition, streptavidin shows very low nonspecific binding. Furthermore, this resin is suitable for gravity flow columns, spin columns, and fast protein liquid chromatography (FPLC) methods. This protocol describes several methods of using GoldBio Ultra HBC™ Streptavidin for purification or immobilization of different biomolecules.

> **Note: These conditions are general guidelines only, and should be optimized according to your application.**

## Materials

- Streptavidin Agarose (GoldBio Catalog # S-105)
- Phosphate Buffered Saline (PBS) (GoldBio Catalog # P-271)
- Spectrophotometer
- Guanidine HCl (GoldBio Catalog # G-211) or
  SDS with Urea (GoldBio Catalog # U-200)
- Glycine HCl
- Tris (GoldBio Catalog # T-400)
- SDS-PAGE Sample Buffer
- Sodium Deoxycholate (GoldBio Catalog # D-070) or
  NP-40 or
  Tween-20
- Triton X-100
- GoldBio Plastic Columns (GoldBio Catalog # P-301 or P-302)

## Method

Column method: Immobilization of biotinylated biomolecules

1. Pour the streptavidin agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of PBS (binding buffer).

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**Note: Ensure that streptavidin-agarose is equilibrated to room temperature before pouring into the column.**

2. Apply your sample containing the biotinylated biomolecule.

3. Wash the biomolecule-bound resin with PBS until the absorbance of the eluate is minimal at 280 nm (< 0.01-0.02).

4. Elute biotinylated biomolecule with 6M guanidine HCl at pH 1.5-2 or by boiling in 2% SDS with 0.4M urea. This will also dissociate streptavidin monomers.

5. Immediately dialyze or desalt eluted samples if needed for downstream applications.

6. Wash the column with 10 column volumes of PBS before use with another antigen.

Batch method: Immunoaffinity purification of proteins (with biotinylated affinity ligand)

1. In a 1.5 ml tube, solubilize antigen in 50 µl of binding buffer (PBS) and add the biotinylated antibody. Adjust the sample volume to 0.2 ml with binding buffer.

2. Incubate the sample for 3-4 hours to overnight at 4°C.

3. Mix the streptavidin agarose resin to ensure an even suspension.

4. Add the appropriate amount of resin to the tube containing the antigen/biotinylated antibody mixture.

5. Incubate the sample by mixing for 1 hour at room temperature or 4°C.

6. Wash the resin-bound complex with 0.5-1.0 ml of binding buffer (PBS). Centrifuge for 1-2 minutes at ~1,000 x g and remove the supernatant.

7. Repeat this wash procedure (step 6) at least four times and remove the final wash.

8. Add elution buffer to the resin to recover the bound antigen. If using 0.1M glycine HCl, pH 2.8, remove the liquid supernatant and immediately adjust the pH by adding a concentrated buffer such as 1M Tris at pH between 7.5 and 9.0 (add 100 µl of this buffer to 1 ml of sample). Alternatively, boil the resin bound complex in SDS-PAGE sample buffer.

Immunoaffinity column (with biotinylated antibody/protein) purification of a protein

1. Pour the streptavidin agarose slurry into an appropriately sized column and wash with 5 to 10 columns of PBS.

2. Apply the biotinylated antibody/protein. Use at least 3 mg of biotinylated antibody per ml of settled streptavidin.

3. Binding of a biotinylated antibody/protein to the streptavidin agarose should be performed at room temperature.

4. Wash the column with PBS until the absorbance of the eluate at 280 nm is less than ~0.01-0.02.

5. Apply the sample (antigen) to the column.

6. Wash the PBS until the absorbance at 280 nm is minimal (0.01-0.02).

7. Elute the sample (antigen) with 0.1M acetic acid or 0.1M glycine HCl (pH 2.5) or other elution buffer to dissociate the antibody-antigen interaction (see Tips section).

8. Immediately neutralize eluted samples with 1M Tris at pH 8.0.

## Tips

- The amount of antigen needed and the incubation time are dependent upon the antibody-antigen system used and may require optimization for each specific system.
- To reduce nonspecific binding, add 1% NP-40, 0.05% Tween 20 or 0.5% sodium deoxycholate to the buffer.
- Use approximately 3 mg of biotinylated antibody/ml of settled streptavidin agarose.
- Prepare biotinylated antibody at 0.2-10 mg/ml in binding buffer (PBS).
- For elution of the biotinylated molecule, use 8M guanidine HCl, pH 2.0 or boil the beads in SDS-PAGE sample buffer.
- PBS, the binding buffer, is composed of 0.1M phosphate and 0.15M sodium chloride at pH 7.2.
- Blocking reagents containing milk products should not be used to block streptavidin agarose due to the presence of endogenous biotin.
- Suggested antibody/antigen elution buffers: 0.1-1.0M glycine, 0.5-1% Triton X-100 at pH 2.5.

## Associated Products

- Streptavidin Agarose (GoldBio Catalog # S-105)
- Phosphate Buffered Saline (PBS) (GoldBio Category # P-271)
- Guanidine HCl (GoldBio Catalog # G-211)
- Urea (GoldBio Catalog # U-200)
- Tris (GoldBio Catalog # T-400)
- Sodium Deoxycholate (GoldBio Catalog # D-070)

## References

Bayer, E. A., Ben-Hur, H., & Wilchek, M. (1990). Isolation and properties of streptavidin. *Avidin-Biotin Technology Methods in Enzymology,* 80-89. Doi:10.1016/0076-6879(90)84262-f.

Chaiet, L. & Wolf, F. J. (1964). The properties of streptavidin, a biotin-binding protein produced by Streptomycetes. *Archives of Biochemistry and Biophysics,* 106, 1-5. Doi:10.1016/0003-9861(64)90150-x.

Gretch, D., Suter, M., & Stinski, M. (1987). The use of biotinylated monoclonal antibodies and streptavidin affinity chromatography to isolate herpesvirus hydrophobic proteins or glycoproteins. *Analytical Biochemistry,* 163(1), 270-277. Doi:10.1016/0003-2697(87)90123-0.

Hoffman, W. L. & Jump, A. A. (1989). Inhibition of the streptavidin-biotin interaction by milk. *Analytical Biochemistry,* 181(2), 318-320. Doi:10.1016/0003-2697(89)90250-9

Weber, P. C., Ohlendorf, D. H., Wendoloski, J. J., & Salemme, F. R. (1989). Structural origins of high-affinity biotin binding to streptavidin. *Science*, 243(4887), 85-88.