

# PROCEDURE FOR USE STREPTAVIDIN RAPID RUN™ Fine AGAROSE BEADS Bulk Resins

## INSTRUCTIONS

These are general guidelines only. Conditions should be optimized for each application.

### 1.- Immobilization of biotinylated biomolecules (Column method)

- Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of PBS.
- Apply your sample containing the biotinylated biomolecule.
- Wash the biomolecule-bound resin with PBS until the absorbance of the eluate is minimal (<0.01–0.02).
- Elute biotinylated biomolecule with 6 M guanidine HCl, pH 1.5–2 or by boiling in 2% SDS with 0.4 M urea. (This will also dissociate streptavidin monomers.)
- Immediately dialyze or desalt eluted samples if needed for downstream applications.

### 2.- Immunoaffinity purification of proteins (with biotinylated affinity ligand) (Batch method)

- In a 1.5 mL tube, solubilize antigen in 50  $\mu$ L of binding buffer (PBS) and add the biotinylated antibody. Adjust the sample volume to 0.2 mL with binding buffer. Incubate sample for 3–4 hr to overnight at 4°C.
- Mix the streptavidin agarose resin to ensure an even suspension. Add the appropriate amount of resin to the tube containing the antigen/biotinylated antibody mixture. Incubate the sample with mixing for 1 hr at room temperature or 4°C.
- Wash the resin-bound complex with 0.5–1.0 mL of binding buffer (PBS). Centrifuge for 1–2 min at  $\sim$ 1,000  $\times$  g and remove the supernatant. Repeat this wash procedure at least four times and remove the final wash.
- Add elution buffer to the resin to recover the bound antigen. If using 0.1 M glycine•HCl, pH 2.8, remove the liquid supernatant and immediately adjust the pH by adding a concentrated buffer such as 1 M Tris, pH 7.5–9.0 (add 100  $\mu$ L of this buffer to 1 mL of sample). Alternatively, boil the resin-bound complex in SDS-PAGE sample buffer.

### 3.- Immunoaffinity column (with biotinylated antibody/protein) purification of a protein

- Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of PBS.
- Apply the biotinylated antibody/protein (use approx. 3 mg (or more) of biotinylated antibody/mL of settled streptavidin agarose).
- Binding of the biotinylated antibody/protein to the streptavidin agarose should be performed at room temperature.
- Wash the column with PBS until the absorbance of the eluate at 280 nm is less than  $\sim$ 0.01–0.02.
- Apply the sample (antigen) to the column.
- Wash with PBS until the absorbance at 280 nm is minimal (0.01–0.02).
- Elute the sample (antigen) with 0.1 M acetic acid or 0.1 M glycine HCl (pH 2.5) or other elution buffer to dissociate the antibody-antigen interaction (see notes).
- Immediately neutralize eluted samples with 1 M Tris, pH 8.0.

## Notes:

- 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.



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- 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 eluting the biotinylated molecule, use 8 M guanidine•HCl, pH 2.0 or boil the beads in SDS-PAGE sample buffer.
- PBS = Binding Buffer = (0.1 M phosphate, 0.15 M sodium chloride, 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 buffer: 0.1–1.0 M glycine, 0.5–1% TritonX-100, pH 2.5.

For laboratory use only. Not for use in diagnostic or therapeutic procedures.