AFFINITY PURIFICATION

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)

a) Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of PBS.
b) Apply your sample containing the biotinylated biomolecule.
c) Wash the biomolecule-bound resin with PBS until the absorbance of the eluate is minimal (<0.01–0.02).
d) 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.)
e) Immediately dialyze or desalt eluted samples if needed for downstream applications.

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

a) 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. Incubate sample for 3–4 hr to overnight at 4°C.
b) 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.
c) Wash the resin-bound complex with 0.5–1.0 mL of binding buffer (PBS). Centrifuge for 1–2 min at ~1,000 × g and remove the supernatant. Repeat this wash procedure at least four times and remove the final wash.
d) 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 µL 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

a) Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of PBS.
b) Apply the biotinylated antibody/protein (use approx. 3 mg (or more) of biotinylated antibody/mL of settled streptavidin agarose).
c) Binding of the biotinylated antibody/protein to the streptavidin agarose should be performed at room temperature.
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performed at room temperature.

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

f) Wash with PBS until the absorbance at 280 nm is minimal (0.01–0.02).

g) 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).

h) 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.
- 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.

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