SUPPLEMENTAL PROTOCOL

Streptavidin Agarose Ultra Performance™

vector LABORATORIES

Together we breakthrough™

Cat. No. **N-1000**

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

Immobilization of biotinylated biomolecules (column method)

1. Pour the streptavidin agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of PBS.
2. Apply the sample containing biotinylated biomolecule to the settled resin bed.
3. Wash the biomolecule-bound resin with PBS until the absorbance of the eluate is nearly zero.
4. Elute biotinylated biomolecule with 6 M guanidine HCl, pH 1.5–2.0, or by boiling in 2% SDS with 0.4 M urea. (Note: This will also dissociate streptavidin monomers.)
5. Immediately neutralize the solution and dialyze or desalt eluted samples if needed for downstream applications.

Immunoaffinity purification of proteins (batch method)

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. Incubate sample for 3–4 hours to overnight at 4°C.
2. 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.
3. Wash the resin-bound complex with 0.5–1.0 ml of binding buffer (PBS). Centrifuge for 1–2 minutes at ~1,000 × g and remove the supernatant. Repeat this wash procedure at least three additional times and remove the final wash.
4. Add elution buffer to the resin to recover the bound antigen. If using 0.1 M glycine HCl, pH 2.5, remove the liquid supernatant and immediately adjust the pH by adding a concentrated buffer such as 1 M Tris, pH 8.0–9.0 (add 100 µl of this buffer to 1 ml of sample). Alternatively, boil the resin-bound complex in SDS-PAGE sample buffer.

Immunoaffinity purification of proteins (column method)

1. Pour the streptavidin agarose slurry into an appropriately sized column and wash with 3 to 5 column volumes of PBS.
2. Apply the biotinylated antibody/protein (use approx. 3 mg of biotinylated antibody/ml of settled streptavidin agarose).
3. Binding of the biotinylated antibody/protein to the streptavidin agarose may be performed at room temperature or 4°C.
4. Wash the column with PBS until the absorbance of the eluate at 280 nm is nearly zero.
5. Apply the sample (antigen) to the column.
6. Wash with PBS until the absorbance at 280 nm is nearly zero.
7. 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).
8. Immediately neutralize the eluted sample with 1 M Tris, pH 9.0.

Notes:

1. The amount of antigen needed, and the incubation time are dependent upon the antibody-antigen system used and may require optimization.
2. To reduce nonspecific binding, add 1% NP-40, 0.05% Tween 20, or 0.5% sodium deoxycholate to the wash buffer.
3. Use approximately 3 mg of biotinylated antibody/ml of settled streptavidin agarose. Prepare biotinylated antibody at 0.2–2.0 mg/ml in binding buffer (PBS).
4. For eluting biotinylated molecules, use 8 M guanidine, pH 2.0, or boil the beads in SDS-PAGE sample buffer. Note: Boiling will dissociate and elute the streptavidin tetramers as well as the antigen.
5. PBS = Binding Buffer = (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2).
6. Blocking reagents containing milk products should not be used to block streptavidin agarose due to the presence of endogenous biotin.
7. Suggested antibody/antigen elution buffer: 0.1–0.2 M glycine, 0.5–1% Triton X-100, pH 2.5.

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