AFFINITY PURIFICATION

PROCEDURE FOR USE
Streptavidin 6HC Agarose Resin

Bulk Resins

INSTRUCTIONS

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

Purification of biotinylated biomolecules

- Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of Binding Buffer (20 mM NaH_2PO_4, 0.15M NaCl, pH 7.4).
- Apply your sample containing the biotinylated biomolecule.
- Wash the biomolecule-bound resin with binding buffer until the absorbance of the eluate is minimal (<0.01–0.02).
- Elute biotinylated biomolecule with 8 M guanidine HCl, pH 1.5
- Immediately dialyze or desalt eluted samples if needed for downstream applications.

Purification of iminobiotinylated biomolecules

- Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of Binding Buffer ($(NH_4)_2\ CO_3$; 0.5M NaCl, pH 10.0).
- Apply your sample containing the iminobiotinylated biomolecule.
- Wash the biomolecule-bound resin with binding buffer until the absorbance of the eluate is minimal (<0.01–0.02).
- Elute iminobiotinylated biomolecule with 50 mM NH_4Ac; 0.5M NaCl, pH 4.0.
- Immediately dialyze or desalt eluted samples if needed for downstream applications.

Purification of antigen with biotinylated antibody

- Pour the streptavidin-agarose slurry into an appropriately sized column and wash with 5 to 10 column volumes of binding buffer (20 mM NaH_2PO_4, 0.15M NaCl, pH 7.4).
- Apply the biotinylated antibody (use approx. 3 mg of biotinylated antibody/mL of settled streptavidin agarose).
- Binding of the biotinylated antibody to the streptavidin agarose should be performed at room temperature.
- Wash the column with binding buffer until the absorbance of the eluate is minimal (<0.01-0.02).
- Apply the sample (antigen) to the column.
- Wash with binding buffer until the absorbance of the eluate is minimal (<0.01-0.02).
- Elute the sample (antigen) with 0.1 M glycine HCL (pH 2.5) or other elution buffer to dissociate the antibody-antigen interaction.
- Immediately neutralize eluted samples with 1 M Tris, pH 8.0.

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