AMID BIOSCIENCES

Streptavidin Agarose Beads

Catalog # SA-101

PROTOCOLS AND INSTRUCTIONS

Streptavidin Agarose is a high affinity biotin-binding chromatography resin prepared by conjugating purified streptavidin to crosslinked 4% agarose beads. Streptavidin agarose is supplied as a 50% suspension in 0.1 M sodium phosphate, pH 7.2, containing 0.15 M NaCl and 0.02% sodium azide. 2 ml of the 50% slurry is equivalent to 1 ml of settled resin.

Sample Protocols

The protocols are provided as examples of applications for this product. Any specific application or task would require optimization of conditions by the user.

Preparation Instructions

The agarose beads must be washed thoroughly with 5 to 10 column volumes of binding buffer (e.g., PBS: 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2) to remove the sodium azide in the storage buffer.

Procedure for Gravity-flow Column Purification of Biotinylated Proteins

1. Equilibrate the streptavidin resin and reagents to room temperature.
2. Pour the streptavidin-agarose into an appropriate column and wash with 5 to 10 column volumes of PBS.
3. Apply the sample containing the biotinylated protein. Incubate the resin with the biotinylated molecule for at least 10 minutes. Reducing incubation time may result in decreased binding capacity.
4. Wash column with 10 column volumes of PBS until the absorbance at 280 nm is minimal.
5. Elute biotinylated proteins using 6 M guanidine-HCl, pH 1.5-2 or by boiling the beads in SDS-PAGE sample buffer. Collect the eluate in 0.5-1 ml fractions. Monitor protein content by measuring the absorbance of each fraction at 280 nm.
   Note: *Proteins biotinylated with 2-iminobiotin can be eluted at non-denaturing conditions. 2-iminobiotin binds tightly to streptavidin at pH ≥9.5 and dissociates at pH 4.*
6. Immediately dialyze or desalt eluted samples if desired.

Procedure for Column Purification of Antigens with Biotinylated Antibody

1. Pour the streptavidin agarose into an appropriate column and wash with 5 to 10 column volumes of PBS.
2. Apply the biotinylated antibody solution to the column and incubate at room temperature for 10 minutes.
3. Wash the column with PBS until the absorbance at 280 nm is minimal.
4. Apply antigen solution to the column.
5. Wash with PBS until the absorbance at 280 nm is minimal.
6. Elute the 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. Monitor protein by measuring the absorbance of each fraction at 280 nm.
7. Immediately neutralize samples eluted at low pH. It is recommended the addition of 0.15 ml of buffer pH 9.0 (e.g. 1M Tris, pH 9.0) per 1 ml of sample.
8. Desalt or dialyze the eluted fractions into a buffer suitable for the downstream application.