Product Information

Streptavidin-Agarose from *Streptomyces avidinii*

Buffered aqueous suspension

S1638

Product Description

Streptavidin is a 66 kDa homotetrameric protein, isolated from *Streptomyces avidinii*, which, like avidin, has a high affinity for biotin (K_a ~ 10^{15} M^{-1}).[1-4] Streptavidin is slightly anionic (pI ~ 5-6) and non-glycosylated. These properties contribute to its relatively low non-specific binding compared to egg white avidin (a glycoprotein with pI ~ 10.5).[1-3,5] Streptavidin is also more resistant than avidin to dissociation into subunits by guanidinium chloride.[6]

Streptavidin-agarose can be used to immobilize or isolate various biotinylated macromolecules and complexes (proteins,[7-9] antibodies,[3,9-12] lectins,[9,13] nucleic acids,[9,14-16] receptors and ligands[9,14]). The inherent high affinity streptavidin-biotin interaction requires harsh conditions to release biotinylated macromolecules. This feature makes streptavidin-agarose useful in a variety of affinity purification applications.[9,14]

This streptavidin-agarose conjugate is prepared by attaching streptavidin to a CNBr-activated 4% agarose through an amino group, to give a 7-atom spacer. Several publications[17-29] and dissertations[30-39] have cited use of S1638 in their research protocols.

Product Profile

This product is provided as a suspension in 0.01 M sodium phosphate, pH 7.2, containing 0.15 M NaCl and 0.02% sodium azide.

Storage/Stability

For long-term storage, store the product at 2-8 °C. **DO NOT FREEZE.** Freezing will damage the bead structure.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The agarose beads must be washed thoroughly with 5-10 column volumes of buffer, to remove the sodium azide in the storage buffer.

Sample Procedures

Note: These procedures are provided as general guidelines. The end user should empirically determine appropriate and optimal conditions for the specific application.

Procedure for Column Purification of Biotinylated Proteins

1. Pour the streptavidin-agarose into an appropriate column. Wash with 5-10 column volumes of PBS.
2. Apply the sample that contains the biotinylated protein.
3. Wash with PBS until the absorbance at 280 nm is minimal.
4. Elute biotinylated proteins either by using 6 M guanidine HCl (pH 1.5-2), or by boiling in 2% SDS with 0.4 M urea.[9]
5. Immediately dialyze or desalt eluted samples, if desired.

Procedure for Immunoaffinity Column Purification of a Protein[3]

1. Pour the streptavidin-agarose into an appropriate column. Wash with 5-10 column volumes of PBS.
2. Apply the biotinylated antibody.
3. Wash the column with PBS until the absorbance at 280 nm is <0.01-0.02.
4. Apply the sample (antigen) to the column.
5. Wash with PBS until the absorbance at 280 nm is minimal.
6. 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.[3,9,16,40] Immediately neutralize the eluted samples.

Notes:

Removal of biotinylated substances from streptavidin-agarose:

- To avoid the harsh and denaturing conditions required to dissociate the biotin-streptavidin interaction, an alternative option is the use of a monomeric avidin-agarose, which requires milder dissociation conditions, such as the use of free biotin or low pH.[41]
- Another option is to iminobiotinylate the protein of interest. Iminobiotin has a pH-dependent interaction with streptavidin which allows tight binding at pH > 9.5 and dissociation at pH 4.[42,43]
- For biotinylated nucleic acid probes, DTT or 2-mercaptoethanol can reportedly be used to disrupt the streptavidin-biotin interaction.[15]

Binding inhibitors:

Non-dialyzed nonfat dried milk, which may contain biotin[44] and various sugars such as mannose,[45] is reported to interfere with biotin binding to streptavidin and avidin.

Reduction of non-specific binding:

If non-specific binding is a problem, consider the following options:

a. Use a different buffer system, or increase the concentration of NaCl to 0.5 M.[46]
b. Increase detergent concentration or use a different detergent.
c. Use a cleared cell lysate, by centrifuging the cell lysate to remove unwanted components.[16]
d. Wash the resin to remove non-specifically bound proteins, such as with a high pH buffer wash (borate buffer, pH 8-9)[46] followed with a low pH buffer wash (acetate buffer, pH 4).
e. Consider using a product with a different charge, such as avidin-agarose or monomeric avidin-agarose.

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