Product Information

ω-Aminohexyl-Agarose

Saline suspension

A6017

Product Description

Hydrophobic ligands can be utilized as bioselective adsorbents. Both ω-Aminoalkyl and alkyl agaroses may interact with regions of hydrophobicity inherent to most proteins. This ω-aminoalkyl agarose product features 1,6-diaminohexane covalently attached via one of its amine groups to activated agarose.

This ω-aminoalkyl agarose product has been used as a parent matrix to attach other ligands for chromatography applications.[1] Several publications have cited use of product A6017 in their research.[2,3]

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.

Storage/Stability

This product is a suspension in 0.5 M NaCl, with preservative. Store at 2-8 °C for long-term storage.

Suggestions for Use

Equilibration Buffers

Hydrophobic Conditions: 0.01 M Tris-HCl (pH 7.0-8.0) plus either:

a) 0.5-1.5 M NaCl, or

b) 1.0-2.0 M $(NH_4)_2SO_4$

Ionic Conditions: 0.01 M Tris-HCl (pH 7.0-8.0)

Other buffer salts may be substituted if the target protein is unstable in Tris buffer. Buffer additions are acceptable and at times essential for protein stability (such as 2-mercaptoethanol or EDTA).

Elution Buffers

Hydrophobic Conditions: Equilibration Buffer (for Hydrophobic Conditions) without NaCl or $(NH_4)_2SO_4$

Ionic Conditions: Equilibration Buffer (for Ionic Conditions) plus either:

a) 0.5-1.0 M NaCl, or

b) 1.0-2.0 M $(NH_4)_2SO_4$

Specific eluants: High salt with the addition of hydrophobic solvents (such as ethylene glycol) or detergents.

Sample Preparation

Centrifugation:

- To eliminate particulates
- Minimize lipid or lipoprotein content (this will aid in resin cleaning and extend column life)

Concentration:

- Between 1-10 mg/mL

Equilibration to column conditions:

- By dialysis
- By desalting columns
- By diafiltration
- By dilution

Procedure

General column chromatography procedure

Recommended running temperature: 2-8 °C

1. Equilibrate each column used with 5-10 column volumes of the appropriate buffer for the target protein.
2. Load the protein solution on the column.
3. Wash the load into the column with a small volume (0.1-0.5 mL) of equilibration buffer.

4. Continue washing with equilibration buffer to remove unbound protein. Washing may require 3-10 column volumes for complete removal of free protein.
5. Elute bound protein with the chosen elution buffer.

   Note: Some proteins may require severe conditions to elute from the column (such as 50% butanol/buffer solutions).
6. Assay elution fractions for the target protein.
7. Evaluate binding capacity vs. total recovery to determine:
   - Maximum binding effectiveness for differing substitutions
   - Maximum recovery
   - Ease of recovery
   - Degree of purification
8. Regenerate the column as directed in the Procedure below.

Regeneration

Wash the column with 10 column volumes each of:

1. 0.05 M NaOH
2. 0.1 M Acetate buffer, pH 4.5
3. Deionized water or distilled water
4. 2.0 M NaCl

Troubleshooting

Problem	Solution
Irreversible binding	Proper choice of ligand length
Denaturing of target protein	1. Rapid post-column treatment (such as desalting columns, diafiltration or dialysis). 2. Utilizing a less hydrophobic column, which would generally require less denaturing elution conditions.

References

1. Hotz, Natascha, "Multiplexed protein caging: Orthogonal and on-command release of angiogenesis-promoting factors". Albert-Ludwigs-Universität Freiburg im Breisgau, Ph.D. dissertation, p. 43 (April 2015).
2. Nakashima, K. *et al.*, *Infect. Immun.*, **65(9)**, 3794-3798 (1997).
3. Li, T.-M. *et al.*, *Sci. Rep.*, **9(1)**, 14226 (2019).

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