Protocol

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Affinity His-Tag Purification Protocol
utilizing Chelating Agarose Bead Spin Columns

Introduction

Recombinant protein purification methods are vital to the advancement of many scientific fields including biochemistry and molecular biology. One powerful method often used to isolate proteins is purification with peptide affinity tags. In this method, recombinant proteins containing a short affinity tag, such as a polyhistidine sequence, are expressed and purified for further studies. Agarose Bead Spin Columns offer a simple way of purifying histidine-tagged protein with no need for any special purification equipment. Pure proteins are obtained quickly and easily with either centrifuge or microcentrifuge tubes with a syringe. Here we describe a protocol for the use of spin columns in the purification of a his-tagged protein.

All GoldBio chelated resins are available in this format.

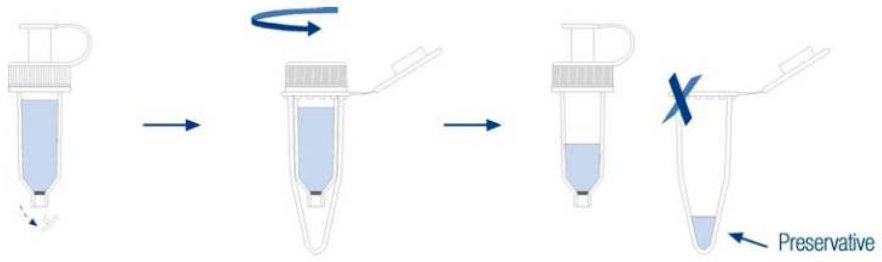
Materials

- GoldBio chelated resins
- Agarose Bead Spin Columns (GoldBio Catalog # P-300)
- Binding buffer containing 20mM disodium phosphate, 500mM NaCl and 10mM imidazole.
- Elution buffer containing 20mM disodium phosphate, 500mM NaCl and 500mM imidazole, at pH 7.5.
- Biological sample

Method

1. To eliminate the preservative, remove the lower cap of the spin column, place in a microcentrifuge tube and centrifuge, then discard the flow-through.

Note: All centrifugation steps carried out in this procedure require mild centrifugation (100-250 x g).



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2. To equilibrate the spin column, place in a microcentrifuge tube and add binding buffer. Centrifuge and discard the flow-through.

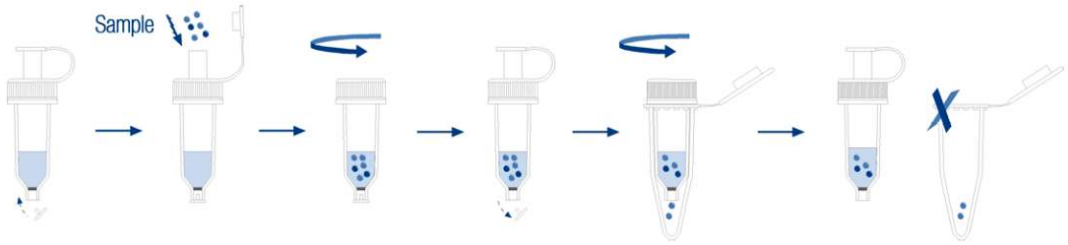
   Note: Generally, binding buffer contains 20mM disodium phosphate, 500mM NaCl and 10mM imidazole, pH 7.5.

   Note: The binding buffer depends on the characteristics of the protein being purified. The most commonly used buffers are acetate (50mM) or phosphate (10-150mM), which have a binding pH close to neutrality (pH 7.0-8.0). However, the binding buffer pH could vary in the following range: 5.5-8.5.

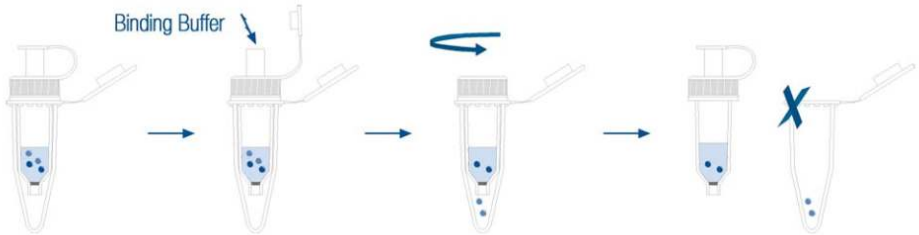
   Note: To avoid ionic interchange effects, add sodium chloride to the binding buffer at a concentration between 0.1M and 0.5M.

   Note: It is also common to add a small amount of imidazole (10-40mM) to improve the selectivity of the binding of the histidine-tagged protein. It is important to use imidazole of high purity to avoid an absorbance increase at 280 nm. It is also important to avoid the presence of reagents such as EDTA or citrate.

3. Add the biological sample containing the histidine-tagged protein. Ensure that the lower cap stays in its place. Manually shake the spin column to maximize contact between the resin and the target-protein. Remove the lower cap, place the spin column in a microcentrifuge tube, and centrifuge (eliminating the proteins not retained in the column).



4. Wash the spin column by placing it in a microcentrifuge tube and adding binding buffer. Centrifuge and discard the flow-through.



5. Elute the protein by adding elution buffer, ensuring the lower cap of the spin column remains in place. Shake manually to drive the elution of the target-protein.

6. Remove the lower cap, place in a microcentrifuge tube and centrifuge, collecting the pure protein in the tube.

Note: Generally, the elution buffer contains 20mM disodium phosphate, 500mM NaCl and 500mM imidazole, at pH 7.5. This concentration of imidazole is usually enough to induce the elution of the target-protein. However, if protein is not eluted, then the concentration may be increased to 2M.

Note: Other reagents that may be used to elute the protein are histidines and ammonium chloride.

Note: Elution may also be performed by decreasing the pH (3.0 or 4.0) or through chelating agents such as EDTA or EGTA (0.05M). The chelating agents induce the desorption of the metal from the resin.

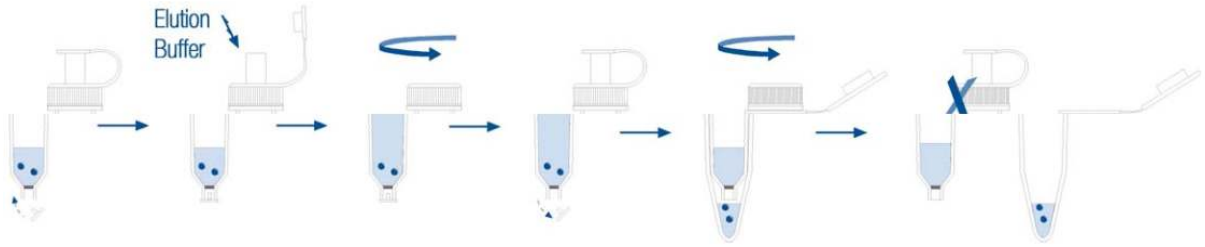


Table 1. Recommendations for affinity-tag protein purification.

Studies	Reagents	
Chemical Stability	HCl 0.01M	SDS 2%
	NaOH 0.1M	2-propanol
	Ethanol 20%	NaOH 1M
	Sodium acetate, pH 4.0	HAc 70%
Denaturing Agents	Urea 8M	Guanidine-HCl 6M
Detergents	Triton X-100 2%	Chaps 1%
	Tween 20 2%	
Additives	Imidazole 2.0M	EDTA 1mM
	Ethanol 20% + glycerol 50%	EDTA 1mM + $MgCl_2$ 10mM
	Na_2SO_4 100mM	Citrate 60mM
	NaCl 1.5M	Citrate 60mM + $MgCl_2$ 80mM

Reducing Agents[a]	Reduced glutathione 10mM B-mercaptoethanol 20mM	DTE 5mM DTT 5mM
Buffers	Na_2HPO_4 50mM, pH 7.5 Tris-HCl 100mM, pH 7.5 MOPS 100mM, pH 7.5	Tris-acetate 100mM, pH 7.5 HEPES 100mM, pH 7.5

[a]Under extended treatments with reducing agents, or in processes where high concentrations of these reagents are used, reduction of the metal ion may result – this will affect the binding capacity of the resin, so these agents should be avoided. The reagents described in the table are compatible with Nickel Activated Agarose Beads (Nickel is most commonly used) under the conditions and concentrations indicated in the table.

Tips

- The use of a mild buffer and imidazole results in purification of biologically active proteins. This is a purification in native conditions.
- If the target protein is insoluble, aggregates, or has a tertiary structure that occludes the histidine tag, then purification must proceed under denaturing conditions.
- The initial cell lysis must be performed using a buffered solution with a pH of 8.0.
- The addition of EDTA in the elution buffer facilitates maximal elution of proteins from the resin. However, this may result in contamination of the eluate since it strips metal away from the resin, which may interfere with enzymatic activity.
- The use of reducing agents is necessary in the case of the presence of untagged proteins with two or more adjacent histidine residues. These proteins may be purified along with the protein of interest, contaminating the eluate.
- Refer to Table 1 for alternatives depending on the type of protein and conditions needed for your purification.

Associated Products

- GoldBio chelated resins
- Agarose Bead Spin Columns (GoldBio Catalog # P-300)

References

Bornhorst, J. A., & Falke, J. J. (2000). Purification of proteins using polyhistidine affinity tags. *Methods in enzymology applications of chimeric genes and hybrid proteins part A: gene expression and protein purification,* 245-254. Doi:10.1016/s0076-6879(00)26058-8.

Kimple, M. E., Brill, A. L., & Pasker, R. L. (2013). Overview of affinity tags for protein purification. *Current Protocols in Protein Science*. Doi:10.1002/0471140864.ps0909s73.

Porath, J. (1992). Immobilized metal ion affinity chromatography. *Protein Expression and Purification, 3*(4), 263-281. Doi:10.1016/1046-5928(92)90001-d.