BTS

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BTS Provides the Best tools for Protein Purification!

AFFINITY HIS-TAG PURIFICATION

PROCEDURE FOR USE CHELATING AGAROSE BEADS Spin Columns

DESCRIPTION

His-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 and microcentrifuge tubes or with a syringe.

All ABT chelated resins are available in this format.

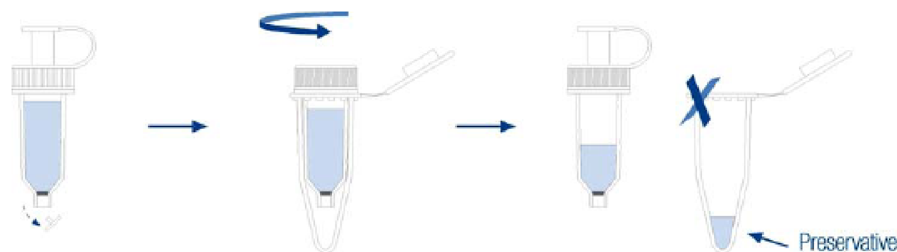
INSTRUCTIONS

The following procedure refers to a protein-tagged purification process under native conditions using the centrifuge. To work under denaturing conditions, first check the stability table at the end. If using a syringe, the process would be performed in an equivalent way.

1. Elimination of the Preservative

Remove the lower cap of the Spin-Column, place in a microtube and centrifuge, then discard the preservative residue collected in the tube.

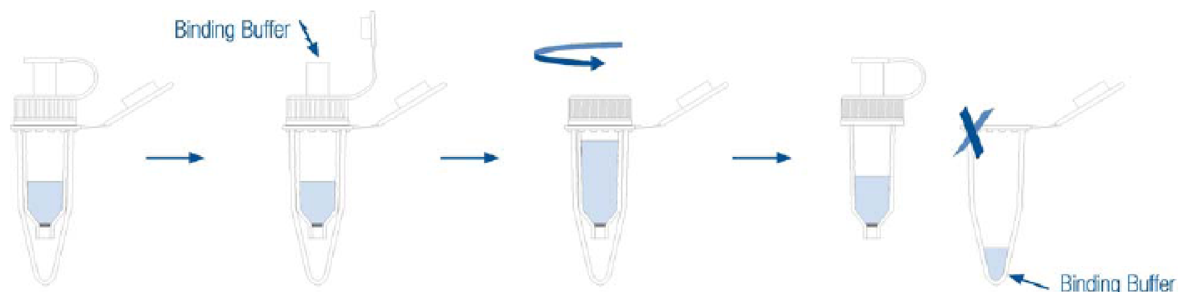
Note: In all centrifugation processes carried out in the procedure, normally a mild centrifugation (1,000 – 1,500 rpm) is sufficient.



2. Equilibration of the Spin-Column

Introduce the Spin-Column in a microcentrifuge tube and add binding buffer through the top. Centrifuge and discard the residue obtained.

Note: As binding buffer, generally 20 mM disodium phosphate, 500 mM NaCl, 10 mM imidazole pH 7.5 is used.



Note: Choosing the binding buffer depends on the characteristics of the protein to be purified. The most commonly used buffers are acetate (50 mM) or phosphate (10-150 mM) which have a binding pH close to neutrality (pH 7.0 – 8.0), however it could vary in the following range: 5.5 – 8.5. To avoid ionic interchange effects, sodium chloride is frequently added to the binding buffer at a concentration between 0.1 and 0.5 M.

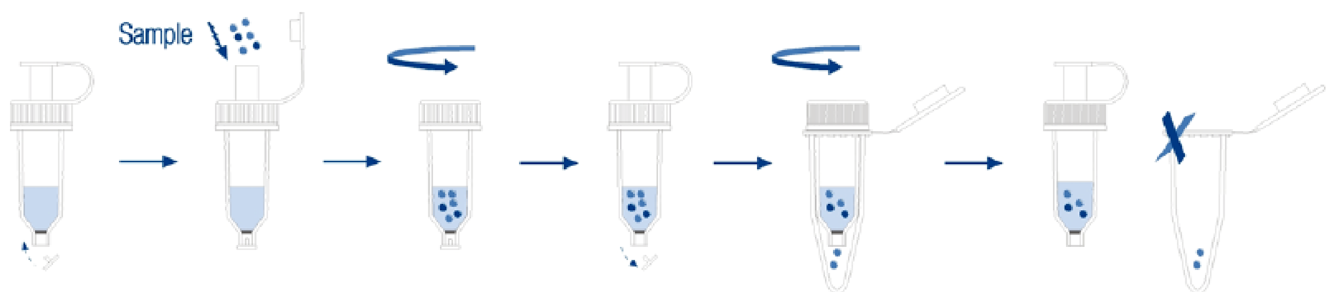
Note: It is also common to add a small amount of imidazole (10 – 40 mM) to improve the selectivity of the binding of the histidine-tagged protein. It is important to use imidazole of high purity to avoid absorbance increases at 280 nm. It is also important to avoid the presence of reagents such as EDTA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

3. Application of the Sample

Add the sample containing the histidine-tagged protein keeping the lower cap in its place.

Manually shake the Spin-Column to maximize contact between the resin and the target-protein.

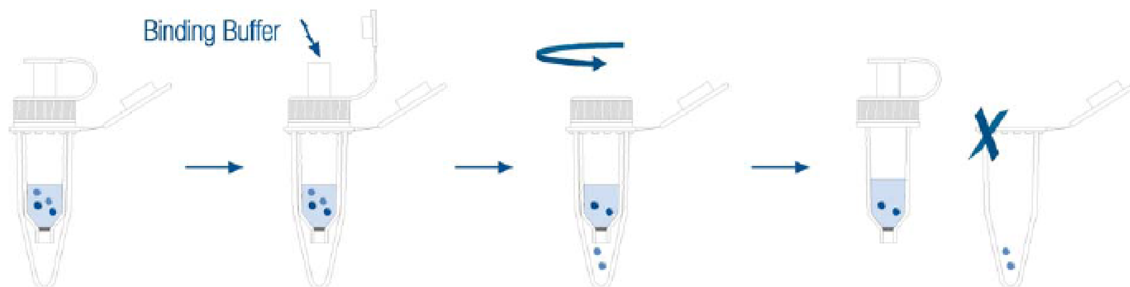
Remove lower cap, introduce the Spin-Column in a microcentrifuge tube and centrifuge (thus eliminating the proteins not retained in the column).



4. Washing of the Spin-Column

Introduce the Spin-Column in a microcentrifuge tube and add the binding buffer through the top.

Centrifuge and discard the residue gathered in the tube.



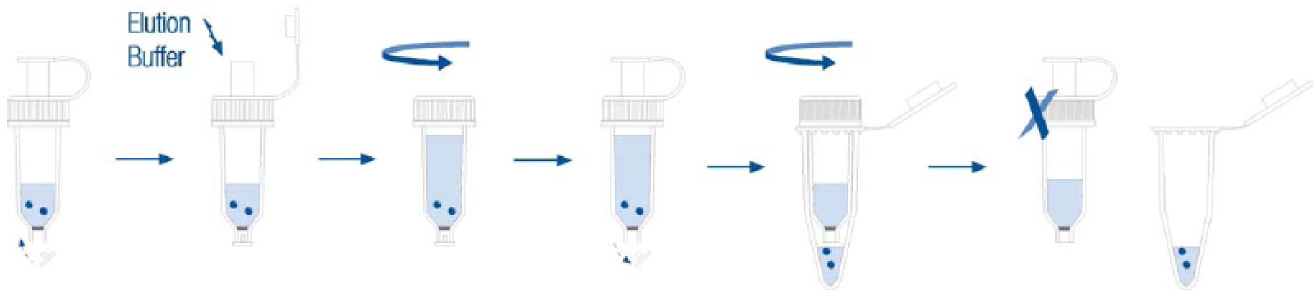
5. Elution of the pure protein

Add the elution buffer with the lower cap of the Spin-Column in place. Manually shake to drive the elution of the target-protein. Remove the lower cap, introduce the Spin-Column in a microcentrifuge tube and centrifuge, finally collecting the pure protein in the tube.

Note: As elution buffer, 20 mM disodium phosphate, 500 mM NaCl, 500 mM imidazole pH 7.5 is generally used. This concentration of imidazole is usually enough to provoke the elution of the target-protein. However if the desired result is not achieved then the concentration may be increased up to 2.0 M.

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

Elution may also be performed by decreasing the pH (3.0 or 4.0) or through chelating agents such as EDTA or EGTA (0.05 M), provoking in this last case the desorption of the metal from the resin.



The recombinant proteins often form inclusion bodies. In these cases the use of denaturing conditions is required:

STUDIES	REAGENTS	
CHEMICAL STABILITY	HCl 0.01 M NaOH 0.1 M Ethanol 20% Sodium acetate pH 4.0	SDS 2% 2-propanol NaOH 1 M HAc 70%
DENATURING AGENTS	Urea 8 M	Guanidine-HCl 6 M
DETERGENTS	Triton X-100 2% Tween 20 2%	Chaps 1%
ADDITIVES	Imidazole 2.0 M Ethanol 20% + glycerol 50% Na_2SO_4 100 Mm NaCl 1.5 M	EDTA 1 mM EDTA 1 mM + $MgCl_2$ 10 mM Citrate 60 mM Citrate 60 mM + $MgCl_2$ 80 mM
REDUCING AGENTS(*)	Reduced glutathione 10 mM ß-mercaptoethanol 20 mM	DTE 5 Mm DTT 5 mM
BUFFERS	Na_2HPO_4 50 mM, pH 7.5 Tris-HCl 100 mM, pH 7.5 MOPS 100 mM, pH 7.5	Tris-acetate 100 mM. pH 7.5 HEPES 100 mM, pH 7.5

(*) Note: 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.

* For laboratory use only. Not for use in diagnostic or therapeutic procedures.