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BTS Provides the Best tools for Protein Purification!

AFFINITY HIS-TAG PURIFICATION

PROCEDURE FOR USE CHELATING AGAROSE BEADS Pre-packed Columns

DESCRIPTION

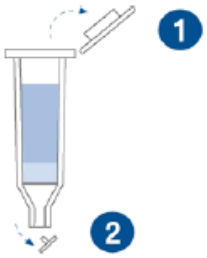
Pre-Packed Columns are in ready-to-use format for purification of histidine-tagged proteins by gravity flow. Rapid purifications and good yield of target proteins are obtainable by this method. All the ABT chelating resins are available in this format.

The resin supplied in the Pre-Packed Columns is suitable for use in both native or denaturing conditions.

This product is supplied as a suspension in 20% ethanol.

INSTRUCTIONS

The following procedure is for the purification of histidine-tagged protein under native conditions. To work under denaturing conditions, first check the stability table below.



1. Elimination of the Preservative

Remove first the upper and then the lower cap of the column, to allow elimination of the preservative by gravity flow.

2. Equilibration of the Pre-Packed Column

Equilibrate the column with 5 - 10 column bed volume of binding buffer. Add the binding buffer on the upper part of the column and make sure no air has been traped. Mix manually inverting the Pre-packed column.

The typical binding buffer is 20 mM disodium phosphate, 500 mM NaCl, 10 mM imidazole at pH 7.5.

Note: Selection of the binding buffer depends on the characteristics of the protein to be purified. The most commonly used buffers are acetate (50 mM) or sodium phosphate (10 – 150 mM). Binding pH is usually close to neutrality (normally pH 7.0 – 8.0), however the larger range 5.5 – 8.5 can be used. To avoid ionic interchange effects, 0.1 - 0.5 M NaCl may be added to the binding buffer.

Note: It is also normal 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 affecting O.D. 280 nm. It is also important to avoid inclusion of reagents such as EDTA or citrate.

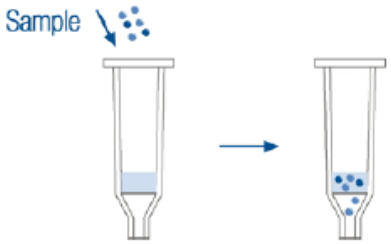
3. Application of the Sample

Add the sample containing the histidine-tagged protein to be purified through the top of the column, keeping sample and resin in contact at least 15 minutes before removing the bottom cap.

Note: In some cases a slight increase of contact time may facilitate binding.

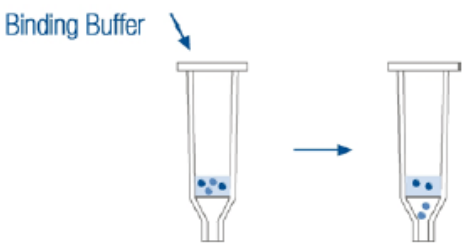
Note: Pouring the sample down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

Note: Binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.



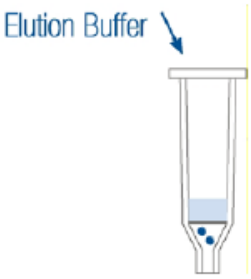
4. Washing of the Pre-Packed Column

Add the binding buffer through the top to eliminate all the proteins that have not been retained in the column. Mix manually inverting the Pre-Packed column. Wash the column with binding buffer until the O.D. 280 nm of the eluent reaches the baseline level.



5. Elution of the Pure protein

Add the elution buffer to the column.



Note: Mix manually inverting the Pre-Packed column. It is advisable to keep elution buffer and resin in contact at least 10 minutes before removing the bottom cap.

Note: Elution buffer is 20 mM disodium phosphate, 500 mM NaCl, 500 mM imidazole at pH 7.5. This imidazole concentration is generally sufficient for elution of the target protein; 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 to 4.0 or 3.0, or with chelating agents such as EDTA or EGTA (0.05 M). However these will also cause desorption of the metal from the resin.

6. Storage

Keep at +2°C - +8°C. Do not freeze.

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.