PROCEDURE FOR USE CHELATING AGAROSE BEADS Test kits

DESCRIPTION

Test kits are a fast and easy way to screen different IMAC resins before choosing the most appropriate product for each target-protein.

All the products supplied in the Test kit are suitable for use in both native or denaturing conditions.

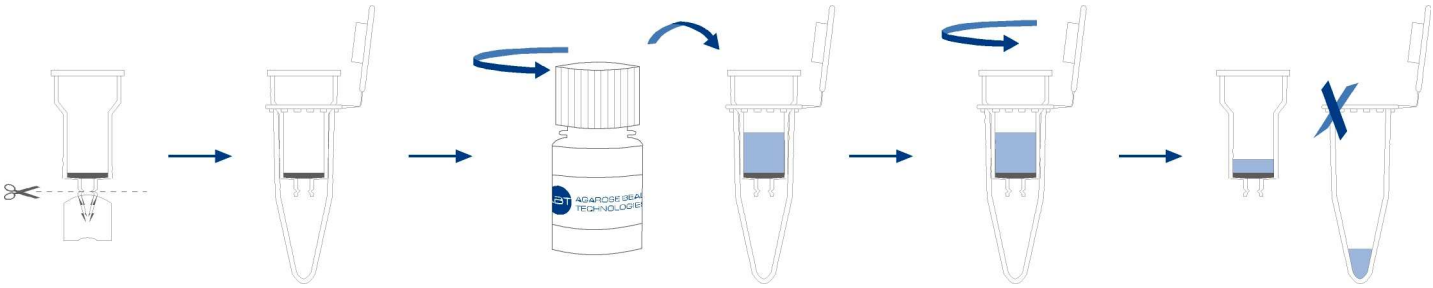
INSTRUCTIONS

The following procedure is for the purification of histidine-tagged proteins under native conditions. For denaturing conditions, consult the stability table at the end.

1. Elimination of the Preservative

Remove the lower cap of the mini-column. Place that mini-column in a microcentrifuge tube. Shake the tester kit containing the resin and add 400 µl suspension to the mini-column. 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.



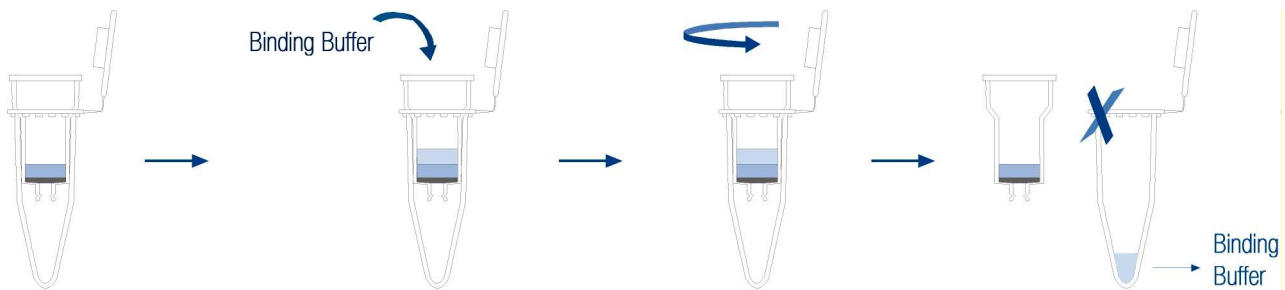
2. Equilibration of the mini-column

Place the mini-column in a microcentrifuge tube and add 1 ml binding buffer through the top. Centrifuge and discard the residue obtained.

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

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 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.

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 absorbance increases at 280 nm. It is also important to avoid inclusion of reagents such as EDTA or citrate.



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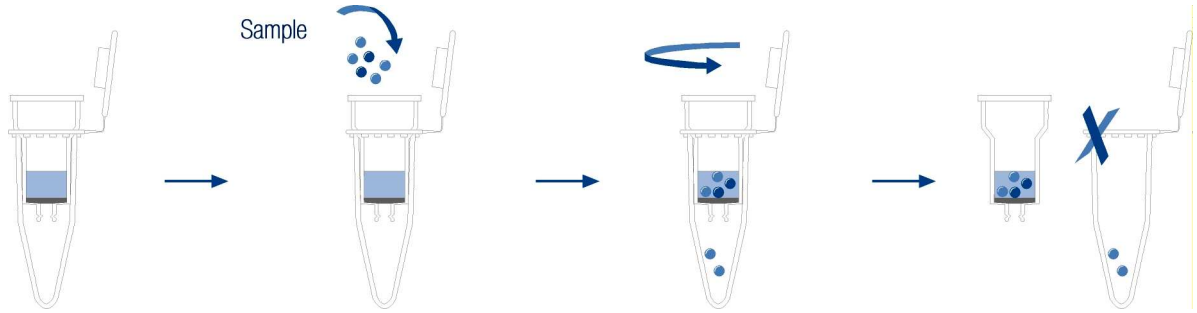
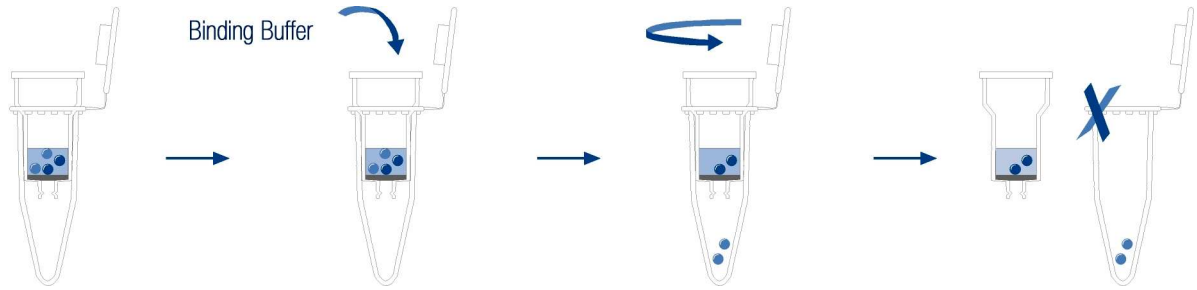
3. Application of the Sample

Place the mini-column in a microcentrifuge tube. Apply the sample with the histidine-tagged protein to be purified and centrifuge (thus eliminating the proteins not retained in the column).



4. Washing of the mini-column

Place the mini-column in a microcentrifuge tube. Add the binding buffer to eliminate all the proteins that have not been retained in the column. Centrifuge and discard the residue obtained.



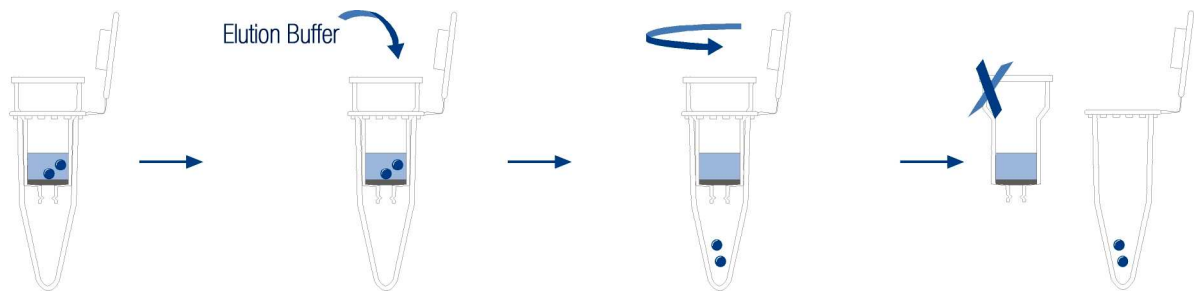
5. Elution of the Pure Protein

Place the mini-column in a microcentrifuge tube. Add the elution buffer to the mini-column. Centrifuge and finally collect the pure protein in the collection tube.

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.

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.



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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. The stability of the Nickel resin has been tested in each of the reagents separately.

There is a very small loss of Nickel with this resin and it only happens in very drastic work circumstances. Given these conditions a slight brown discoloration may appear but does not usually affect performance. The discoloration is due to small particles of Nickel breaking away from the main body of resin and coming into contact with the reducing agents causing the Nickel to reduce and became brown in color. If there is a risk of producing this effect, it can be avoided by pre-treatment of the resin before it has ever been used. This treatment eliminates the cations that are weakly attached to the resin before beginning the process, so the reducing agent doesn't affect the lost Nickel ions.

Pre-treatment:

1. - Wash the resin with five column volumes of distilled water.
2. - Wash the resin with five column volumes of binding Buffer (without reducing agents in the buffer).
3. - Wash with five column volumes of elution buffer (without reducing agents in the buffer).
4. - Equilibrate with 10 column volumes of binding buffer (without reducing agents).

Once this treatment has been carried out, the resin is ready for the simple purification in such circumstances as described above.

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

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