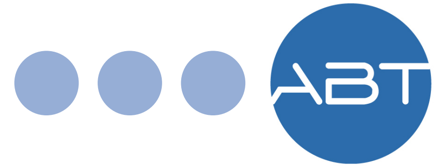

PROCEDURE FOR USE
NICKEL Affinity Cartridges (5ml)

DESCRIPTION

NICKEL Affinity Cartridges 5ml are used for purification of histidine-tagged proteins in native or denaturing conditions. This cartridge can be used with an automated chromatography system, a peristaltic pump or with a syringe for manual processing.

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

INSTRUCTIONS

Cartridges (5 ml) can be operated with liquid chromatography systems (such us ÄKTA™ design systems) via standard 10-32 fittings without additional connectors. The recommended flow rate is 5ml/min.

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. Connect Cartridge to the chromatography system

Purge the pump with binding buffer. Assure that all air is displaced. Remove the snap-off end at the cartridge outlet and save it for further use. Remove the upper plug from the cartridge.

Fill the inlet port of the cartridge with several drops of buffer to remove air to form a positive meniscus. Start the pump and insert the fitting "drop-to-drop" into the cartridge port to avoid introducing air bubbles.

Wash the beads with at least 25 ml of distilled water to eliminate the preservative.

2. Equilibration of the Cartridge

Equilibrate the cartridge with at least 25 ml of binding buffer. The typical binding buffer is 20 mM sodium 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 7.5 – 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

Once the cartridge is equilibrated, add the filtered or centrifuged sample containing the histidine-tagged protein to be purified through the top of the cartridge.
Note: Filter the sample through a 0.45 µm filter and/or centrifuge it immediately before the application to the cartridge.
Note: Binding capacity was tested using purified Dehydroxyacetone (6 x His) and the result was 110 mg DHAK-(6x His) purified / ml medium. This is only an indicative value because binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.
Note: In some cases a slight increase of contact time may facilitate binding.

4. Washing of the Cartridge

Wash the cartridge with binding buffer until the O.D. 280 nm is stable.

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5. Elution of the pure protein

Add the elution buffer to the cartridge using a one-step (e.g. 25 ml) or linear gradient (e.g. 100 ml).

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.

Note: Conditions (volumes, times, temperatures) used for elution may vary. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted protein)

6. Storage

Before storage, it is recommended to wash the cartridge with at least 25 ml of 20% ethanol.

Keep at +2ºC - +8ºC. 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 Rapid Run^{TM} Cartridges 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.

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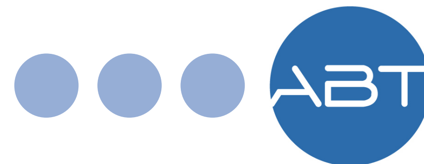

PROCEDURE FOR USE
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Pre-treatment:

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 50 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.

NICKEL AFFINITY CARTRIDGES REGENERATION PROCEDURE

During the life of the resin, it may lose binding points because some protein is retained. Hence a loss of the binding capacity may be observed in successive cycles. To return to the starting state, regeneration may be necessary. Regeneration consists of the complete elimination of the metal and therefore of the retained protein.

In general, cartridge regeneration is always necessary when changing proteins. When continuing with the same protein it is recommended to do regeneration when an appreciable diminution in the yield is observed. The frequency of these stages varies with the protein and the conditions used.

A. Elimination of the metal from the resin: it is necessary to wash the resin with at least 25 ml of a solution 20 mM sodium phosphate containing 0.5 M NaCl, 50 mM of EDTA at pH 7.4.

B. Elimination of the excess EDTA: In order to eliminate the residual EDTA before reloading the resin with the corresponding metal, the cartridge should be washed with at least 25 ml of binding buffer and finally at least 25 ml of distilled water.

Note: In some special cases it is advisable to make more drastic intermediate stages of regeneration to eliminate denatured proteins or lipids.

Drastic Regeneration Stage: denatured proteins and lipids elimination.

Use when denatured proteins or lipids may have been retained after stage B.

i. Elimination of ionic interactions: Wash with 1.5 M NaCl (at least 50 ml). Later wash with 50 ml of distilled water to eliminate ions.

ii. Elimination of precipitated proteins (may be responsible for cartridge pressure changes). Wash the cartridge with 1.0 M NaOH at least during 2 hours. Eliminate the NaOH with 50 ml of binding buffer followed by 50 ml of distilled water.

iii. Elimination of strong hydrophobic interactions: wash the cartridge with a solution of isopropanol 30% for approximately 30 minutes. Then wash with 50 ml of distilled water to eliminate the isopropanol.

iv. Wash the cartridge with 50 ml of a solution 0.5% of non-ionic acetic acid detergent 0.1 M. Rinse the detergent with ethanol 70% (approximately 50 ml). Finally wash with 50 ml of distilled water to rinse out the ethanol.

C. Load the cartridge with the corresponding metal: once the excess EDTA has been eliminated, add at least 25 ml of the 0.1 M metal solution (normally chlorides or sulphates are used).

D. Elimination of the excess of metal: wash with at least 25 ml of distilled water.

E. Preparation of the column: add at least 25 ml of the binding buffer.

Note: If the cartridge is not going to be used for a while it is recommended to replace the last step by the addition of the preservative.
Also it is recommended to include a regeneration step when beginning to purify a new protein.

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

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