



## High Density Nickel Agarose

Cat. No.	Amount
AC-303-25	25 ml
AC-303-100	100 ml
AC-303-500	500 ml

### For research use only!

**Shipping:** shipped at ambient temperature

**Storage Conditions:** store at 4 °C

**Additional Storage Conditions:** do not freeze

**Shelf Life:** 12 months after date of delivery

**Form:** supplied as a suspension in 20 % ethanol

### Description:

Metal-containing affinity resins are designed for batch or column purification of His-tagged proteins.

### General Procedure for the Purification of His-tagged Proteins

The following procedure describes the purification of His-tagged proteins under native conditions. The affinity of the protein to the resin will depend on first at all the chemistry of the resin (number of chelate groups, chelating metal), second the accessibility of the His-tag, and third pH and composition of the buffer.

#### 1. Removal of the storage solution

Wash the beads with 5 - 10 column volumes of distilled water to eliminate the storage solution.

**Please note:** In case of Metal Free Agaroses (AC-301, AC-302), the column must be loaded with metal prior to use, as described in the following steps:

- Prepare a 0.1 M solution (pH < 7.0) of the salt (chloride or sulphate) of the required metal ( $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ ,  $Co^{2+}$ ).
- Add 5 column volumes of this salt solution to the column.
- Wash the agarose with 5 - 10 column volumes of distilled water to eliminate non-retained metal.

#### 2. Equilibration

Equilibrate the column with 5 - 10 column volumes of binding buffer. The choice of binding buffer depends on the particular properties of the protein as well as on the type of chelate used. The most frequently used buffers are acetate (50 mM) or phosphate (10 - 150 mM). The pH of binding buffers is usually close to neutral pH (7.0 - 8.0), but can vary over the range 5.5 - 8.5. To avoid ionic interchange, add 0.15 - 0.5 M of NaCl.

**Please note:** In some cases addition of imidazole (10 - 40 mM) to the binding buffer will increase the selectivity of protein binding. Usage of high purity imidazole will avoid affecting OD<sub>280</sub> measurements. Avoid the presence of chelating agents such as EDTA or citrate during the complete purification process.

#### 3. Sample loading

Once the resin is equilibrated, the sample containing the tagged protein for purification is applied. In some cases, a slight increase of contact time may facilitate binding.

**Please note:** Binding capacity can be affected by several factors, such as sample concentration, binding buffer or the flow rate during sample application.

#### 4. Wash

Wash the resin with the binding buffer until OD<sub>280</sub> nm reaches baseline level.

#### 5. Elution

Elution of the purified protein can be performed in several ways:



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- Addition of a competitive ligand (usually imidazole), which allows the elution of the retained protein. In general, 0.5 M of imidazole is sufficient to elute the protein. It is also possible to apply a concentration gradient of imidazole (0 - 0.5 M). Most proteins are eluted at imidazole concentrations around 250 mM. Other reagents that can be used as competitive ligands are histidine and ammonium chloride.

Please note: Usually, subsequent elimination of imidazole from the purified protein is not necessary. It can however, be removed by dialysis, precipitation with ammonium sulphate or ultrafiltration.

- Reduction of the pH (with or without gradient) of the elution buffer also allows the elution of the desired protein (between pH 3.0 and 4.0).
- A more drastic method uses reagents such as EDTA or EGTA (0.05 M), which causes the elution of both the protein and the chelating metal.

**Please note:** Milder elution can be achieved by using a different affinity resin with lower interaction with the protein (different chelating metal ions with lower affinity).

Recombinant proteins often form insoluble inclusion bodies which have to be rendered soluble by a purification under denaturing conditions, e.g. by using 7 M urea or 6 M guanidine chloride during the purification process.

### Column Packing

The following procedure describes how a column is packed from bulk affinity resins:

- Shake the bottle manually to obtain a homogenous suspension of the affinity resin. Place a funnel in the column head and slowly pour the suspension down the walls of the column.  
**Please note:** Add the suspension slowly to avoid the formation of bubbles. The suspension may also be degassed before being applied to the column.  
Decant the product and discard most of the leftover liquid, leaving 1 cm above the column head to prevent drying out. This can be done either by passing the liquid through the column, or pipetting it from the top of the column.
- Repeat the previous step until the desired column height is obtained.
- Insert the adapter gently into the column head until it begins to displace the liquid.  
**Please note:** Make sure no air is trapped under the net.
- Add distilled water via the tubing until a constant height (corresponding to the height of the column) is achieved.  
**Please note:** If the desired height is not achieved, repeat steps 1 through 4.
- Once a constant height has been achieved, rinse the column with 5 volumes of distilled water for complete elimination of the storage solution.
- Equilibrate the column with 5 - 10 column volumes of binding

buffer.

**Please note:** It is recommended to de-gas all solutions prior to adding them to the column to avoid the formation of bubbles.

### Recommended Conditions

Linear flow rate: 26 cm/h

Recommended flow rate: 0.5 - 1.0 ml/min

Max. pressure: 2.6 psi (0.18 bar)

### Regeneration of Chelating Resins

Binding capacity of chelating resins may decrease after several cycles of usage, because some protein may have been retained. To revert binding capacity to the starting state, regeneration may be necessary. The regeneration procedure will completely remove the metal and therefore also any retained protein.

In general, column regeneration is always recommended when beginning to purify a new protein. When continuing with the same protein, it is recommended to perform a regeneration when an appreciable diminution of the yield is observed. The frequency of these stages varies with the protein and the conditions used.

- Elimination of the metal from the resin  
Wash the resin with 5 column volumes of a solution containing 20 mM sodium phosphate, 0.5 M NaCl and 50 mM EDTA at pH 7.0.
- Elimination of excess EDTA  
In order to eliminate residual EDTA before reloading the resin with the corresponding metal, the column is washed with 5 column volumes of distilled water.  
**Please note:** If denatured proteins or lipids may have been retained after this step, it is recommended to regenerate the resin under more drastic conditions:
  - Elimination of ionic interactions: Wash in batch for approx. 20 minutes in 1.5 M NaCl. Subsequently wash with 10 column volumes of distilled water to eliminate ions.
  - Elimination of precipitated proteins: Precipitated proteins can be responsible for column pressure changes. Wash in batch for at least 2 hours in 1.0 M NaOH. Eliminate the NaOH with 10 column volumes of distilled water.
  - Elimination of strong hydrophobic interactions: Resuspend the resin in batch in 30 % isopropanol for approx. 20 minutes. Then wash with 10 column volumes of distilled water to eliminate the isopropanol.
  - Wash in batch for 2 hours with 0.5 % of a 0.1 M solution of a non-ionic acetic acid detergent. Rinse the detergent with 70 % ethanol (approximately 10 column volumes). Finally wash with 10 column volumes of distilled water to rinse out the ethanol.
- Loading of the column with the corresponding metal:  
Once the excess of EDTA has been eliminated, add 5 volumes



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of the 0.1 M metal solution (usually chlorides or sulphates are used). Wash with 5 column volumes of distilled water to remove the excess of metal ions.

- Equilibration of the column:  
Add 5 column volumes of the binding buffer.  
Please note: If the resin is not going to be used for a while it is recommended to replace the last step by the addition of the storage solution.

### Selected References:

Gasset-Rosa *et al.* (2008). Negative regulation of pPS10 plasmid replication: origin pairing by zipping-up DNA-bound RepA monomers. *Mol. Microbiol.* **68**:560.

Giraldo (2007). Defined DNA sequences promote the assembly of a bacterial protein into distinct amyloid nanostructures. *Proc. Natl. Acad. Sci. USA* **104**:17388.

Bastida *et al.* (2003) In Vivo Chaperone-Assisted Folding of 1,6-Fucosyltransferase from *Rhizobium sp.* *Chem Bio Chem* **4**:531.

Yip *et al.* (1994) Immobilized Metal Ion Affinity Chromatography. *Molecular Biotechnology* **1**:151.

Anspach *et al.* (1994). Silica-based metal chelate affinity sorbents. I. Preparation and characterization of iminodiacetic acid affinity sorbents prepared via different immobilization techniques. *Journal of Chromatography A* **672**:35.

Porath (1992) Immobilized Metal Ion Affinity Chromatography. *Protein Expression and Purification* **3**:263.

Porath *et al.* (1991) Cascade-mode multi-affinity chromatography. Fraction of human serum protein. *Journal of Chromatography* **550**:751.

Hemdan *et al.* (1985) Development of immobilized metal affinity chromatography. II: Interaction of amino acids with immobilized Nickel Iminodiacetate. *Journal of Chromatography* **323**:255.

Porath *et al.* (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature* **258**:598.