Nickel Beads

Cat. 1018-5/10/25

His-tag affinity purification gel

Ver 1.4 03.07

Introduction

Recombinant proteins tagged with 6-10 poly Histidines can be purified in one step by ion metal affinity chromatography (IMAC). Several metal ions exhibiting affinity to poly-Histidines are commonly used, Nickel and Cobalt being the most popular. Tagging the proteins may be done at the N-ter or the C-ter by cloning a gene in dedicated expression vectors encoding to the poly Histidines stretch, enabling the purification of the recombinant protein resulted.

Adar's Nickel Beads are high quality Nickel affinity-resin product, that exhibit high capacity and good selectivity towards His-tagged proteins. Purification with **Adar's Nickel Beads** may be done in a variety of formats, such as gravity-flow columns and small or large-scale batches. The preferred purification strategy is to start by using the native purification conditions and only later on, if required, use denaturing conditions for proteins that are accumulated as inclusion bodies or in cases when the 6xHis purification tag is not exposed in the native form. The amount of actual protein bound can vary with the type and size of protein. Reducing agents such as DTT (Dithiothreitol) and chelating agents such as EDTA (Ethylene diamine tetra acetic acid) and EGTA (Ethylene glycol-bis(beta-amino-ethyl ether)), may be used in the buffers employed in the extraction protocols, but not during the affinity purification itself. Gel filtration or dialysis is recommended procedures for removal of these agents.

Nickel Beads Specifications

Matrix: Sepharose CL-4B
Activation method: Oxiran.
Chelating group: Iminodiacetic acid
Binding capacity: ~6-9 mg pure (His) 6 -tagged protein (Mr ~ 49 000) per ml
Mean bead size: 40-165 µm
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm2
Recommended flow rate: 1-2 ml/min/cm2
Stability of the matrix: pH 2-11.
Storage: 4°C in PBS pH 7.4 added with NaN_3 0.1% (w/v) or 20% ethanol as preservatives

Protocol: Protein Purification under Native Conditions

A. Buffers needed

Lysis buffer (5 ml): Your choice of lysis buffer (preferably one that does not contain reducing agents and chelators).
Regeneration buffer (100 ml): 0.02 M sodium phosphate, 0.5 M NaCl, pH 7.4, EDTA 50mM.
Nickel solution (10 ml): 5 mg/ml $NiSO_4 \cdot 6H_2O$ in ddH_2O
Double distilled water (100 ml).
Binding buffer (100 ml): 0.02 M sodium phosphate, 0.5 M NaCl, pH 7.4
Elution buffer (200 ml): 0.02 M sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

B. Cell lysis

1. Thaw frozen cells pellet and resuspend in 5 ml of Lysis buffer of your preference (not supplied). Bacterial culture volume processed for purification may be between 50-250 ml in volume. Avoid lysis buffers containing reducing agents or chelators. Alternatively, use such buffers and dialyze the lysate or apply gel filtration technique to remove reducing agents or chelators from the lysate. Mix thoroughly to create homogenate. Add imidazole to final concentration of 5 mM, for inhibiting non-tagged contaminating proteins binding to **Nickel Beads**. (imidazole concentration may be reduced to 1 mM if binding of the target protein to is weak.
2. Sonicate or homogenize on ice to lyse cells (i.e. six 10 sec long sonication cycles with 5 sec pauses between).
3. Centrifuge lysate at 10,000 x g for 20 min at 4°C. Collect supernatant. Save 20 µl of the supernatant for SDS-PAGE analysis.

C. Purification

1. Mix 5 ml of the nickel-chelate slurry thoroughly until homogeneous suspension is visible. Transfer the gel suspension into an appropriate column with inner diameter of 1.0 to 1.5 cm.
2. After column preparation equilibrate the column with Binding buffer by washing with 5-10 column volumes. Recommended flow rates are 1-2 ml/min/cm^2.
3. Apply the sample to column at a rate between 0.1 ml/min to 0.5 ml/min using a syringe or a pump. A partial displacement of chelated nickel ions is often noted as the protein is adsorbed (the blue color is changed to off-white color). The total volume of the sample applied is not critical in most cases. Save the flow through for SDS-PAGE analysis.
4. Wash with 5-10 column volumes Binding buffer (not supplied). To increase the purity of eluted protein a wash with Binding buffer containing 5-50 mM imidazole is often effective. If the protein of interest is present in lysate supernatants but has not bound well to the column the imidazole concentration added to lysate should be restricted to 1mM final concentration in order to improve binding.
5. Elute with Elution buffer (not supplied), at flow rates of 1-2 ml/min/cm^2. Two to five column volumes are usually needed for elution of the 6xHis tagged protein. A gradient type of elution where imidiazole concentration ranges between 0 and 500 mM may be used to improve the separation of the target protein from contaminants. A linear gradient with 10-20 column volumes is recommended. Typically, imidazole concentrations of 100 mM to 200 mM are sufficient to elute most 6xHis proteins. 500 mM imidazole has A280 ~0.5 (5 mm cell). Use the Elution buffer as blank when quantitation of the target protein in eluted fractions.

D. Re-equilibration and Storage

1. Strip the column by washing it with 10 column volumes of Regeneration buffer.
2. Wash the column with 10 volumes of distilled water.
3. Prepare 5 mg/ml $NiSO_4 \cdot 6H_2O$ in ddH_2O. Apply the $NiSO_4$ solution on the column at a flow rate of 1-2 ml/min/cm^2 until saturation.
4. Wash the column with distilled water with 3-5 volumes of distilled water.
5. Equilibrate the column with the Binding buffer.
6. Storage conditions: equilibrate the column with the Binding buffer added with NaN_3 0.1% (w/v) or 20% ethanol as preservatives.