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HisPur Purification of His-Tagged Proteins--CHEM 584

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Abstract

The Thermo Scientific HisPur Ni-NTA Resin enables effective immobilized metal affinity chromatography (IMAC) purification of polyhistidine-tagged proteins from a soluble protein extract. This resin is composed of nickel-charged nitrilotriacetic acid (NTA) chelate immobilized onto 6% crosslinked agarose. The Ni-NTA resin is compatible with native or denaturing conditions and can be used in multiple formats, including conventional gravity-flow chromatography, spin column and FPLC. Ni-NTA resins are commonly chosen for His-tagged-protein purification because of the four metal-binding sites on the chelate, which allow for high-binding capacity and low-metal ion leaching.

Guidelines

Important Product Information

• Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein. Therefore, it is important to optimize these parameters before attempting a large-scale purification. For best results, perform a small-scale test to estimate the expression level and determine the solubility of each His-tagged protein.

• Optimization of the lysis procedure is critical for maximizing protein yield. Some methods for protein extraction include using B-PER Bacterial Protein Extraction Reagent, mechanical methods such as freeze/thaw cycles, sonication or French press.

• Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8M urea, 6M guanidine) and purified with the Ni-NTA resin, but a denaturant must be added to buffers so the protein remains soluble throughout the procedure.

• These instructions are effective for many types of samples; however, optimization might be required to further reduce nonspecific binding. To optimize conditions, adjust the recommended imidazole concentration in the Equilibration, Wash and Elution Buffer.

• Avoid using protease inhibitors or other additives that contain chelators, such as EDTA, or strong reducing agents, such as DTT or β-mercaptoethanol, which will disrupt the function of the nickel resin.

• When using the Bradford Assay to monitor protein concentration in the elution fractions, dilute the samples at least 1:2 before performing the protein assay.

Materials

For native conditions prepare the following buffers:

- Equilibration Buffer: 20mM sodium phosphate, 300mM sodium chloride (PBS) with 10mM imidazole; pH 7.4
- Wash Buffer: PBS with 25mM imidazole; pH 7.4
- Elution Buffer: PBS with 250mM imidazole; pH 7.4

For denaturing conditions prepare the following buffers:

- Equilibration Buffer: PBS with 6M guanidine•HCl and 10mM imidazole; pH 7.4
- Wash Buffer: PBS with 6M guanidine•HCl and 25mM imidazole; pH 7.4
- Elution Buffer: PBS with 6M guanidine•HCl and 250mM imidazole; pH 7.4

For resin regeneration prepare the following buffer:

- MES Buffer: 20mM 2-(N-morpholine)-ethanesulfonic acid, 0.1M sodium chloride; pH 5.0

1 Add 250 µL of a 50% Ni-NTA resinslurry to a 1.7 ml microcentrifuge tube. Centrifuge tube for 00:02:00 at 700 × g and carefully remove and discard the supernatant.

Note

The HisPur Ni-NTA Resin allows for purification strategy customization. Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4°C.

It is important to spin the resin only at speeds up to 700 x g

You should only use a 1 ml pipette tip when manipulating the resin.

2 Add two resin-bed volumes of Equilibration Buffer and mix gently until the resin is fully resuspended

Note

Since you added 250 µL of the 50% resin slurry to your tube, your resin bed volume is 125 µL

3 Centrifuge tube for 00:02:00 at 700 × g and carefully remove and discard buffer.

4 Prepare sample by mixing the protein extract from your B-PER Lysis with an equal volume of Equilibration Buffer. The total volume should equal at least two volumes of the resin bed.

5 Add the prepared protein extract to the tube and mix on an end-over-end rotator for 30 minutes.

6 Centrifuge the tube for 00:02:00 at 700 × g. If desired, save supernatant for downstream analysis.

7 Wash the resin with two resin-bed volumes of Wash Buffer. Centrifuge the tube for 00:02:00 at 700 × g. Save supernatant for downstream analysis.

8 Repeat wash step. Optional: You can monitor the washing by measuring the absorbance of the supernatant by at 280 nm until a baseline is reached.

9 Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer. Centrifuge tube for 00:02:00 at 700 × g. Carefully remove and save the supernatant. Repeat this step twice, saving each supernatant fraction in a separate tube.

10 Monitor protein elution by measuring the absorbance of the fractions at 280nm. Since you are purifying a fluorescent protein, you can also monitor the protein concentration by measuring the absorbance at 500 nm.

Note

The eluted protein can be directly analyzed by SDS-PAGE. Samples containing 6M guanidine•HCl must be dialyzed against a buffer containing 8M urea before SDS-PAGE analysis.