Propure™ His Tag Ni-NTA Purification Kit

Cat #: D-AKTP201

Size: 1 mL/1 mL*5

Storage: Stable for 12 months at 4°C

Assay Principle

Propure™ His Tag Ni-NTA Resin which can withstand severe conditions such as reducing agent, denaturant or coupling agent with a certain concentration, and can purify His-tag protein simply, quickly, efficiently and with high specificity. Propure™ His Tag 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 4% crosslinked agarose. 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.

Materials Supplied and Storage Conditions

Kit components	Size（1 mL）	Size（1 mL*5）	Storage conditions
His-Tag Purification Nickel Column	1 mL	1 mL*5	4°C
Phosphate-Buffered Saline (PBS,10×)	30 mL	100 mL+50 mL	4°C
Imidazole (2 M)	25 mL	125 mL	4°C

Materials Required but Not Supplied

- 0.22 μm or 0.45 μm filter
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Various glassware for preparing reagents and buffer solutions

Sample Preparation

I-A Protein extraction from bacteria or yeast

1. Transfer bacteria or yeast culture medium to centrifugal tube after induced protein expression, centrifuge 15 min at 7,000 rpm to collect bacteria or yeast.

2. Mix with 10 times volume of Lysis buffer (collected bacteria or yeast:Lysis buffer=1:10), add PMSF with final concentration 1mM. Lysozyme is recommended to be added with a final concentration of 0.2-0.4 mg/mL.

3. Resuspend the cells. Add 10 μg/ml RNase A and 5μg/ml DNase I in case the cells' concentration is high. Mix and sonicate the suspension in ice, keep the lystes to be clean.

4. Transfer the clean lysate to a new centrifuge tube, centrifuge for 20-30 min at 10,000 rpm, 4°C. Take the supernatant, store on ice or -20°C.

I-B Soluble protein extraction from yeast, insect or mammalian cells

1. Transfer cell culture medium to centrifugal tube, centrifuge 10 min at 5,000 rpm to collect suspension. Suspension should be dialyzed with Lysis buffer in case there're EDTA, histidine and other reductants in suspension.

2. Large volume of suspension should be concentrated by Ammonium sulfate fractional precipitation method, following be dialyzed with Lysis buffer.

I-C Inclusion-body protein extraction for purification in denaturing conditions

1. Transfer culture medium to centrifugal tube, centrifuge 15 min at 7,000 rpm to collect precipitation. Remove the suspension.

2. Mix with 10 times volume of Lysis buffer, such as collected precipitation: Lysis buffer= 1:10 (W/V). Lysis buffer does not contain 8 M Urea. Resuspend the precipitation. Mix and sonicate the suspension in ice.

3. Transfer the lysate to a new centrifuge tube, centrifuge for 20-30 min at 10,000 rpm, 4°C. Dispose the supernatant, Repeat step 2 and 3 once again.

4. Mix with 10 times volume of Lysis buffer, such as collected precipitation: Lysis buffer=1:10 (W/V). Resuspend the lysate for purification in denaturing conditions.

Reagent Preparation

It is recommended to filter all buffers before use by passing through a 0.22 μm or 0.45 μm filter. For most proteins, the following buffer are recommended:

Reagent	10× PBS (mL)	2 M Imidazole (μL)	Water (mL)
Lysis buffer	1	50	8.95
Wash buffer	1	125	8.875
Elution buffer	1	1250	7.75

Note: Sometimes overexpressed proteins are sequestered in inclusion bodies. Inclusion bodies of His-tagged proteins can be solubilized in 8M urea or 6M guanidine. We recommend you use the buffer below (Reagents below are not supplied in the kit)：

For imidazole elution method prepare the following buffers:

Lysis buffer: 8 M Urea, 50 mM NaH_2PO_4 , 300 mM NaCl, 10 mM imidazole, pH 8.0;

Wash buffer: 8 M Urea, 50 mM NaH_2PO_4 , 300 mM NaCl, 20 mM imidazole, pH 8.0;

Elution buffer: 8 M Urea, 50 mM NaH_2PO_4 , 300 mM NaCl, 250 mM imidazole, pH 8.0.

For pH elution method prepare the following buffers:

Lysis buffer: 8 M Urea, 100 mM NaH_2PO_4, 100 mM Tris·HCl, pH 8.0;

Wash buffer: 8 M Urea, 100 mM NaH_2PO_4, 100 mM Tris·HCl, pH 6.3;

Elution buffer: 8 M Urea, 100 mM NaH_2PO_4 , 100 mM Tris·HCl, pH 4.5.

Sample Purification Procedure

1. Fix Column. Move the top and bottom stopper, and let the storage buffer drain away.

2. Add 5 resin-bed volume Lysis Buffer to the column. Equilibrate the column (make the Ni-NTA Resin in the same buffer system as the target protein to protect the protein). Allow buffer to drain from the column.

3. Add the prepared protein extract to the resin. (In order to improve the recovery rate of the target protein, the adding speed was controlled to ensure the full contact between the target protein and Ni 2^{+}).

Note: Collect the flow-through which can be analyzed by SDS-PAGE. When problems arise, it is easier to find solutions.

4. Add 10-15 resin-bed volume Wash Buffer to the column to remove the non-specific adsorption protein. Pay attention to collecting the flow-through.

5. Add 5-10 resin-bed volume Elution Buffer to the column to wash the target protein. The collected eluate is the target protein solution.

6. Add 3 resin-bed volume Lysis Buffer and 5 resin-bed volume deionized water to the column in turn to equilibrate the Ni-NTA Resin. Store resin in an equal volume of PBS containing 20% ethanol at 4-30°Cto prevent the resin from being contaminated by bacteria.

7. The flow-through, eluted protein and prepared protein extract can be directly analyzed by SDS-PAGE to test the purification effect.

Cleaning-in-Place(CIP)

When the back pressure is too high or obvious contamination appears on the resin during the use of the resin, it needs to be cleaned-in-place (CIP). It is recommended to follow the steps below to remove residual contaminants on the resin, such as precipitated proteins, hydrophobins, and lipoproteins.

To remove strongly bound hydrophobic proteins, lipoproteins and lipids:

Wash the column using 5-10 resin-bed volumes of 30% isopropanol contacting for 15-20 min. Or apply 2 resin-bed volumes of acidic or alkaline solution containing detergent (i.e. 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent), for 1-2 hours. Finally wash the column with 10 resin-bed volumes of distilled water.

To remove the proteins engaged with ionic interaction:

Wash the column with 1.5 M NaCl for 10-15 min. Finally wash the column with 10 resin-bed volumes of distilled water.

Ni-NTA ResinRegeneration

In general, The Ni-NTA resin may be used at least five times before it becomes necessary to recharge them with metal ions.When the back pressure is too high or the capacity significantly lower, it needs to strip the metal ions and recharge

the resin as the following procedure:

1. Wash resin with 5 resin-bed volumes of deionized water;

2. 100 mM EDTA(pH 8.0), 5 resin-bed volumes;

3. Wash resin with 10 resin-bed volumes of deionized water;

4. Wash resin with 5 resin-bed volumes of 0.5 M NaOH and stay for 10-15 min;

5. Wash resin with 10 resin-bed volumes of deionized water;

6. 100mM $NiSO_4$, 3-5 resin-bed volumes;

7. Wash resin with 10 resin-bed volumes of deionized water;

After regeneration, the medium can be used immediately or store in PBS containing 20% ethanol at 4°C.

Chemical Compatibilities

Reducing agents	5 mM DTE, 0.5-1 mM DTT, 20 mM β-mercaptoethanol, 5 mM TCEP, 10 mM reduced glutathione
Denaturing agents	8 M urea, 6 M Gua-HCl
Detergents	2% Triton X-100 (nonionic), 2% Tween 20 (nonionic), 2% NP-40 (nonionic), 2% cholate (anionic), 1% CHAPS (zwitterionic)
Other additives	500 mM imidazole, 20% ethanol, 50% glycerol, 100 mM Na2SO4, 1.5 M NaCl, 1 mM EDTA, 60 mM citrate
Commonly used buffer	50 mM sodium phosphate, pH 7.4, 100 mM Tris-HCl, pH 7.4, 100 mM Tris-acetate, pH 7.4, 100 mM HEPES, pH 7.4, 100 mM MOPS, pH 7.4, 100 mM sodium acetate, pH 4

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.