HisPur Ni NTA Kit

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1. Product Description

Ni NTA Beads can be used to purify 6xHis-tagged proteins expressed in series of expression vectors, such as *E.coli*., yeast, insect cells and mammalian cells. Ni NTA Beads consists of 90μm beads of 4% agarose, to which Nitilotriacetic acid (NTA) has been coupled. The chelating group has then been charged with nickel ions (Ni^{2+}). This form is very stable octahedral structure of nickel ions in the center, which can protect the nickel ions from attack of the competitive small molecule. The structure of Ni-NTA is compatible with a certain concentration of reducing agents, denaturing agents, detergents and other additives. Ni NTA Beads is highly stable and expand the range of suitable operating conditions.

HisPur Ni NTA kit contains one HisPur Ni NTA Column, Lysis Buffer and Elution Buffer. See table 3. It is fast, simple and easy operation.

Table 1. Characteristics of **Ni NTA Beads**

Item	Description
Matrix Spherical	4% agarose
Static Binding Capacity	＞40mg 6×His-tagged protein/ml medium
Particle size	45-165μm
Maximum Pressure	0.1MPa, 1bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	4-30℃

Table 2. Chemical compatibilities for **Ni NTA Beads**

Reagent	Stability
Reductants	5 mM DTE 0.5-1mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione
Denaturants	8 M urea 6 M Gua-HCl
Detergent	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 Na_2SO_4 1.5 M NaCl 1 mM EDTA 60 mM citrate
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

Table 3. The component of **HisPur Ni NTA Kit**

	Cat.No	Size	Amount
HisPur Ni NTA Column	SA004K-1	3ml	1
Lysis Buffer	SA004K-2	100ml	2
Elution Buffer	SA004K-3	100ml	1

2. Purification Procedure

2.1 Sample Preparation

2.1.1 Recombinant native protein expressed in *E.coli* or yeast

1) Single colonies were cultured in LB medium. According to the instruction, adding the inducers for a period of time.

2) Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000rpm(7,500×g) for 10-15min at 4℃. Discard supernatant and determine weight of pellet. Resuspend pellet in 1:10 ration (w/v) in lysis buffer and add lysozyme (0.2-0.4mg/ml cell paste, if the host cell containing pLysS or pLysE, it can be without lysozyme) and PMSF (1mM/ml cell paste).

3) If high concentration of cell suspension, it is consider to add 10μg/ml RNase A and 5μg/ml DNase I. Sonicate the cell suspension/lysate on ice.

4) Centrifuge the homogenized lysate at 10,000rpm(15,000×g) for 20min at 4℃ to clarify sample. Save supernatant.

2.1.2 Native protein expressed in yeast, insect or mammalian cells

1) Harvest the cells from an appropriate volume of culture by centrifugation at 5,000rpm(3,800×g) for 10-15min at 4℃. Save supernatant. If the supernatant without EDTA, histidine and reductant, it can be purified directly, otherwise it need dialysis to 1XPBS under 4℃ .

2) for a large volume of supernatant, it need precipitation by adding ammonium sulfate and dialysis to1XPBS under 4℃.

2.1.3 Inclusion bodies from *E.coli*

1) Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000rpm(7,500×g) for 10-15min at 4℃. Discard supernatant and determine weight of pellet.

2) Resuspend pellet in 1:10 ration (w/v) with Lysis Buffer. Sonicate the cell suspension/lysate on ice.

3) Centrifuge the homogenized sample at 10,000rpm(15,000×g) for 20min at 4℃ to pellet the inclusion.

4) Resuspend pellet in 1:10 ration (w/v) with denaturing Lysis Buffer(containing 8M urea). Sonicate, as needed, to redissolve the pellet.

5) Analyze the concentration of the target protein and continue with purification protocols under denaturing conditions.

2.2 Column Purification

HisPur Ni NTA kit contains HisPur Ni NTA Column, Lysis Buffer and Elution Buffer. The purification process takes about 30min. It mainly depends on the sample volume and solution viscosity.

1). Fix HisPur Ni NTA Column and remove the bottom plug and top plug. Allow storage buffer to drain from resin by gravity flow.

2). Equilibrate column with 5ml Lysis Buffer. Allow buffer to drain from the column. Repeat this step two times.

3). Add the prepared protein extract to the resin. Collect the flow-through. If desired, re-apply the flow-through once to maximize binding.

4). Wash resin with 5ml Lysis Buffer and collect the flow-through. Repeat this step five times or repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.

5). Elute His-tagged proteins from the resin with 15-30ml Elution Buffer. Collect the fraction every 5ml in a separate tube.

6). Equilibrate the column with 5ml Lysis Buffer and 5ml deionized water alternately. Repeat 2 times. Then Equilibrate the column with 5ml 20% ethanol. Finally store the beads with 1XPBS containing 20% ethanol at 2-8℃.

Note: To remove imidazole for downstream applications use gel filtration or dialysis.

2.3 Analysis

Identify the fractions containing the His-tagged protein. Use UV absorbance, SDS-PAGE, or western blot.

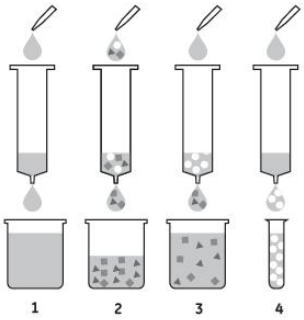

Fig.2. Purification process of HisPur Ni NTA Column

Step1：Equilibrate；Step2：Binding；Step3：Wash；Step4：Elution

3. Cleaning-in-Place

A column used to purify protein from cell extract usually contains some soluble substances and cell debris that are nonspecifically absorbed onto the matrix. Cleaning-in-Place eliminates material not removed by regeneration and prevents progressive buildup of contaminants. If the column is to be reused, these contaminants should be cleaned from the column, as they were not completely removed during the sample clarification steps.

Remove the strong hydrophobic binding protein, lipoprotein and lipid

Wash the column using 5-10 column volumes 30% isopropanol contacting for 15-20min. Or you can choose the 2CV acidic or alkaline solution containing detergents, for example, 0.1 M acetic acid solution contains 0.1-0.5% non-ionic detergent, contacting for 1-2 hours . Finally wash the column with 10CV distilled water

Remove the proteins combined with ion interacting

Wash the column with 1.5M NaCl solution contacting for 10-15min.

Finally wash the column with 10 column volumes distilled water.

4. Troubleshooting

Problem	Probable Cause	Solution
Back pressure exceeds 3 bar	Column is clogged	Cleaning in place(part 3).
		Increase the centrifugation speed or filtering the sample.
	Sample is too viscous	Increase sonication or add DNase I (5 μg/ml with 1mM Mg2+. Incubate on ice for 15min.
	Buffer is too viscous	Dilute sample by adding more homogenization buffer.
No protein is eluted	Expression of target protein in extract is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply large sample volume.
	Target protein is found in the flow through	Reduce imidazole concentration in lysis buffer sample and wash buffer. Or increase buffer pH.
	Elution conditions are too mild.	Increase imidazole concentration in Elution buffer. Or decrease buffer pH.
		Strip nickel ion by using 10-100mM EDTA solution, at the same time you can obtain target protein.
	Protein degradation or purification cause the histidine tag to be removed.	Operate at 4°C. Add protease inhibitors.
		Make a new construct with his-tag attached to other terminus.
His-tagged protein is not pure	Wash is not enough	Increase the volume of Wash Buffer.
	Association between the his tagged protein and protein contaminant.	Optimize the wash condition by adjusting the pH and imidazole concentration.
		Add an additional chromatography step, that is ion exchange, hydrophobic interaction or size exclusion.
The medium are brown.	The buffer contains DTT.	Use Ni NTA Beads or Ni NTA Beads FF when the reductant concentration bellow 2mM.
Protein precipitates during purification	Temperature is too low	Perform the purification at room time.
	Aggregate formation	Add solubilization agents to the samples and buffers, for example 0.1% Triton X-100 , Tween-20 and ≤20% glycerol to maintain protein solubility.

5. Related Products

Product	Cat. No.	Size
Ni NTA Beads	SA004005	5 ml
	SA004025	25 ml
	SA004100	100 ml
	SA004500	500 ml
	SA00401L	1 L
	SA00410L	10 L
HisPur Ni NTA Kit	SA004K03	3 times
Ni NTA Beads 6FF	SA005005	5 ml
	SA005025	25 ml
	SA005100	100 ml
	SA005500	500 ml
	SA00501L	1 L
	SA00510L	10 L
HisCap 6FF	SA005C11	1×1 ml
	SA005C51	5×1 ml
	SA005C15	1×5 ml
	SA005C55	5×5 ml
	SA005CS	3×1 ml+1×5 ml