Ni NTA Beads

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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 90 μm beads of 4% agarose, to which nitrilotriacetic 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 (See Fig 1.). The structure of **Ni NTA Beads** is compatible with a certain concentration of reducing agents, denaturing agents, detergents and other additives (See Table 2). **Ni NTA Beads** has wider applicability, more stable ligand and higher selectivity than that of Ni IDA Beads.

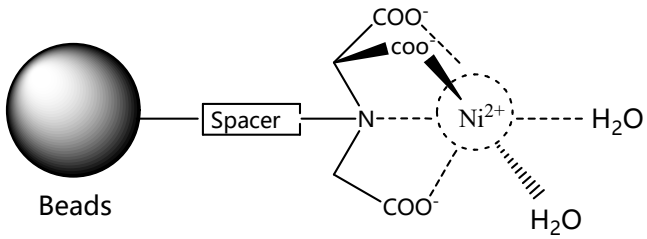

Fig.1. The chemical structures of Ni NTA Beads

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

Item	Description
Matrix Spherical	4% agarose
Static Binding Capacity	＞40 mg 6×His-tagged protein/ml medium
Particle size	45-165 μm
Maximum Pressure	0.1 MPa, 1 bar
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)

Table 2. Chemical compatibilities for **Ni NTA Beads** (Continued table)

Reagent	Stability
Other additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA 60 mM citrate

2. Purification Procedure

2.1 Buffer Preparation

The basic principle of the following recommended buffer and other buffer is low concentration of imidazole in Lysis and wash buffer and high in elution buffer. Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.22 μm or 0.45 μm filter before use. **Ni NTA Beads** can be used for the his-tagged protein purification under native conditions and denaturing conditions, which need different buffer. See table 3, table 4 and table 5.

Table 3. Recommended buffer for his-tagged protein purification under native conditions

Name	Volume	Ingredient
Lysis Buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 10 mM imidazole (0.68 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution.
Wash Buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 20 mM imidazole (1.36 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution.
Elution Buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 250 mM imidazole (17.0 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution.

Table 4. Recommended buffer for his-tagged protein purification under denaturing conditions, pH elution

Name	Volume	Ingredient
Lysis Buffer	1 L	8 M Urea (480.50 g Urea) 100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 100 mM Tris·HCl (15.76 g Tris·HCl) Adjust the buffer pH to 8.0 with HCl solution
Wash Buffer	1 L	8 M Urea (480.50 g Urea) 100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 100 mM Tris·HCl (15.76 g Tris·HCl) Adjust the buffer pH to 6.3 with HCl solution
Elution Buffer	1 L	8 M Urea (480.50 g Urea) 100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 100 mM Tris·HCl (15.76 g Tris·HCl) Adjust the buffer pH to 4.5 with HCl solution

Table 5. Recommended buffer for his-tagged protein purification under denaturing conditions, imidazole elution

Name	Volume	Ingredient
Lysis Buffer	1 L	8 M Urea (480.50 g Urea) 50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 10 mM imidazole (0.68 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution.
Wash Buffer	1 L	8 M Urea (480.50 g Urea) 50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 20 mM imidazole (1.36 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution.
Elution Buffer	1 L	8 M Urea (480.50 g Urea) 50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) 250 mM imidazole (17.0 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution.

Note: 8 M Urea could also be replaced by 6 M Guanidine-Hcl. Table 4 or Table 5 select one of them as the solution for the inclusion body purification.

2.2 Sample Preparation

2.2.1 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 (0.2-0.4 mg/ml cell paste, if the host cell containing pLysS or pLysE, it can be without lysozyme) and PMSF (1 mM/ml cell paste).

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

4) Centrifuge the homogenized lysate at 10,000 rpm for 20 to 30 min at 4℃ to clarify sample. Save supernatant.

2.2.2 Native protein expressed in yeast, insect or mammalian cells

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

2) For a large volume of supernatant, it need precipitation by adding ammonium sulfate and dialysis to 1×PBS under 4℃.

2.2.3 Inclusion bodies from *E.coli*

1) Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000 rpm 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 10,000 rpm or 20 min at 4℃ to pellet the inclusion.

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

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

2.3 Column Purification

1) Mix the slurry by gently inverting the bottle several times to completely suspend the **Ni NTA Beads.** Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.

2) Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Lysis Buffer onto the column to equilibrate the beads.

3) Apply the sample to the column and drain the flow-through. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.

4) Wash the column with 10-15 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.

5) Elute the sample with 5-10 column volumes Elution Buffer and collect the eluate.

6) Equilibrate the column with 3 column volumes of Lysis Buffer , 5 column volumes of distilled water and 5 column volumes of 1X PBS containing 20% ethanol. Finally store the resin with 1×PBS containing 20% ethanol at 4℃.

2.4 Analysis

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

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 with 5-10 column volumes 30% isopropanol contacting for 15-20 min. Or you can choose the 2 column volumes 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 10 column volumes distilled water.

Remove the proteins combined with ion interacting

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

Finally wash the column with 10 column volumes distilled water.

4. Regenerating the medium

In general, **Ni NTA Beads** may be used a number of times before it becomes necessary to recharge them with metal ions. When the back pressure is too highor the capacity significantly lower, it need to strip the metal ions and recharge the **Ni NTA Beads** as the following procedure.

Wash the column with one of the following solutions.

1) Rinse with 5 column volumes of distilled water;

2) 100 mM EDTA (pH 8.0), 5 column volumes;

3) Rinse with 10 column volumes of distilled water;

4) 0.5 M NaOH, 5 column volumes, contacting for 10-15 min;

5) Rinse with 10 column volumes of distilled water;

6) 100 mM $NiSO_4$, 3-5 column volumes;

7) Rinse with 10 column volumes of distilled water;

After regeneration, the medium can be used immediately, otherwise, it need to be suspended in an equal volume of 1×PBS containing 20% ethanol at 4℃.

5. 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 Mg^{2+}. Incubate on ice for 15 min.
		Dilute sample with Binding buffer.
No protein is eluted	Protein may be an inclusion body, not in the supernatant.	The lysate can be analyzed by electrophoresis for the presence of the target protein in the supernatant, and the inclusion body protein need to be purified according to the purification method of the inclusion body protein.
	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 sample lysis and wash buffer. Or increase the pH of buffer.
	Protein degradation or purification cause the histidine tag to be removed.	Operate at 4℃. Add protease inhibitors.
		Make a new construct with his-tag attached to other terminus.

(Continued table)

Problem	Probable Cause	Solution
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 color of medium becomes shallow.	The nickel ions was stripped.	Chelate nickel ions according to the part 4.
The color of medium becomes brown	The buffer contains reducing agent.	Refer to Table 2 to reduce the concentration of reducing agent
Protein precipitates during purification	Temperature is too high	Perform the purification at 4℃.
	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.

6. 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