Ni NTA Magarose Beads

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1. Product Description

Ni NTA Magarose 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. 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.

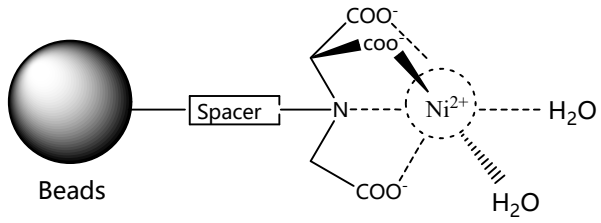

Fig.1. The chemical structures of Ni NTA Magarose Beads

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

Item	Description
Matrix Spherical	Magnetic agarose
Static Binding Capacity	＞40 mg 6×His-tagged protein/ml medium
Particle size（μm）	30-100 μm
Beads concentration	20%(V/V) slurry
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	2-8℃

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

Reagent	Stability
Reductants	5 mM DTE 0.5-1 mM 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

2. Purification Procedure

2.1 Buffer Preparation

The basic principle of the following recommended buffer and other buffer is lower concentration of imidazole in Lysis and wash buffer and higher in elution buffer. Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.22 μm or 0.45 μm filter before use. **Ni NTA Magarose Beads** can be used for the his-tagged protein purification under native conditions and denaturing conditions, which need different buffer. The recommended buffer is shown in 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 NaH2PO4 （7.80 g NaH2PO4·2H2O） 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 $NaH_2PO_4·2H_2O$） 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 $NaH_2PO_4·2H_2O$） 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(elute by pH)

Name	**Volume**	**Ingredient**
Lysis Buffer	1 L	8 M Urea （480.50 g urea） 100 mM NaH_2PO_4 （15.60 g $NaH_2PO_4·2H_2O$） 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 $NaH_2PO_4·2H_2O$） 100 mM Tris·HCl （15.76 g Tris·HCl） Adjust the buffer pH to 6.3with HCl solution
Elution Buffer	1 L	8 M Urea（480.50 g urea） 100 mM NaH_2PO_4（15.60 g $NaH_2PO_4·2H_2O$） 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(elute by imidazole)

Name	**Volume**	**Ingredient**
Lysis Buffer	1 L	8 M Urea （480.50 g urea） 50 mM NaH2PO4 （7.80 g NaH2PO4·2H2O） 300 mM NaCl （17.54 g NaCl） 10 mM imidazole （0.68 g imidazole） Adjust the buffer pH to 8.0 with HCl solution
Wash Buffer	1 L	8 M Urea （480.50 g urea ） 50 mM NaH2PO4 （7.80 g NaH2PO4·2H2O） 300 mM NaCl （17.54 g NaCl） 20 mM imidazole（1.36 g imidazole） Adjust the buffer pH to 6.3with HCl solution
Elution Buffer	1 L	8 M Urea（480.50 g urea） 50 mM NaH2PO4 （7.80 g NaH2PO4·2H2O） 300 mM NaCl （17.54 g NaCl） 250 mM imidazole （17.0 g imidazole） Adjust the buffer pH to 4.5 with HCl solution

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 7,000 rpm(7,500×g) for 10-15 min 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 high,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 10,000 rpm(15,000×g) for 20 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(3,800×g) 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 1XPBS solution under 4℃ .
2) for a large volume of supernatant, it need precipitation by adding ammonium sulfate and dialysis to1XPBS solution 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(7,500×g) for 10-15 min 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 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 Preparation of the Magnetic Beads

The protocol uses 100 μl **Ni NTA Magarose Beads**, but this may be scaled up or down as required.
1) Completely resuspend the beads by shaking or vortexing the vial.
2) Transfer 500 μl **Ni NTA Magarose Beads** (20% v/v) into a clean tube.
3) Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4) Add 1 ml Lysis Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.

2.4 Protein Purification

1) Transfer the 6X His-tagged Protein lysate to **Ni NTA Magarose Beads.** Incubate the tube at room temperature with mixing (on a shaker or rotator) for 10-30 min. Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant. If necessary, keep the supernatant for analysis.
2) Add 1 ml Wash Buffer to the tube and mix well, use the magnetic separation rack to collect the beads and discard the supernatant. Repeat the wash step three more times.
3) Add 200-300 μl Elution Buffer, mix well and incubation for 5-10min. Use the magnetic separation rack to collect the beads and transfer the supernatant into a new tube. Repeat this step one more time.

2.5 Analysis

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

3. Additional Information

1) Please read the product instruction carefully before using the product.
2) In the process of magnetic beads preservation, operations such as freezing, drying and high-speed centrifugation should be avoided, otherwise the structure of magnetic beads will be damaged and the binding capacity of proteins will be seriously affected.
3) Before using the magnetic beads, please oscillate gently and fully to keep the beads in a uniform suspension state.
4) The baeds can be reused to purify the same protein; when different proteins are purified, it is recommended to use new magnetic beads to avoid cross-contamination.

4. Related Products

Product	Cat. No.	Size
Ni IDA Magarose Beads	SM001001	1 ml
	SM001005	5 ml
	SM001025	25 ml
	SM001100	100 ml
	SM00101L	1 L
Ni NTA Magarose Beads	SM008001	1 ml
	SM008005	5 ml
	SM008025	25 ml
	SM008100	100 ml
	SM00801L	1 L
Ni Smart Magarose Beads	SM025001	1 ml
	SM025005	5 ml
	SM025025	25 ml
	SM025100	100 ml
	SM0501L	1 L