High-Affinity Ni-NTA Resin

Technical Manual No. 0237 **Version 20070418**

I. DESCRIPTION

High Affinity Ni-NTA Resin (Cat. No. L00250, 25 ml as 50 ml of 50% slurry) is an agarose resin (4% cross-linked) covalently coupled to a tridentate chelating agent (NTA) that binds Ni^{2+} ions by four coordination sites for high-affinity purification of His-tagged recombinant proteins without leacking of Ni^{2+}. His-tagged proteins may be purified under either native or denaturing conditions from any of the common recombinant expression systems, such as bacteria, yeast, insect, and mammalian. Proteins bound to the resin are then eluted with either low pH buffer or imidazole solution or even with histidine solution.

II. KEY FEATURES

- High Binding capacity: more than 20 mg of 6xHis-tagged protein (50 kD) /ml (CV).
- Compatible with various reagents needed in the purification process, see table 1.
- pH stability of 3-13 (short term 2-14).
- Resin can be regenerated for multiple uses.

Table 1. Reagents Compatible with High Affinity Ni-NTA

Denaturants	Detergents	Reducing agents	Others	Salts
6 M Gu·HCl	2% Triton X-100	20 mM β-ME	50% glycerol	4 M $MgCl_2$
8 M Urea	2% Tween 20	30 mM DTT	20% ethanol	5 mM $CaCl_2$
	1% CHAPS		1 mM EDTA	2 M NaCl

III. HIS-TAGGED FUSION PROTEIN PURIFICATION PROCEDURE

1. Purification of polyhistidine-tagged proteins under native conditions

Before use, prepare the following three Buffers:

Lysis-Equilibration Buffer (LE buffer, 1 liter):
• 50 mM NaH2PO4
• 300 mM NaCl
• Adjust pH to 8.0 using NaOH

Wash Buffer
• 50 mM NaH2PO4
•300 mM NaCl
•10 mM imidazole
• Adjust pH to 8.0 using NaOH

Elution buffer (1 liter):
• 50 mM NaH2PO4
• 300 mM NaCl
• 250 mM imidazole
• Adjust pH to 8.0 using NaOH

(1). Sample preparation and pretreatment to remove large particles and high concentration of reagents such as EDTA, amino acids and reducing agents, which can destroy Ni-NTA resins.

A. For protein expressed in *E. coli* or yeast cytoplasm

a) Harvest cells from a 50 ml culture by centrifugation (*e.g.*, 5,000 rpm for five minutes in a Sorvall SS-34 rotor). Resuspend the cells in 8 ml of LE buffer with appropriate amount of PMSF or other protease inhibitors. The inhibitors must have no effect on the ability of the Ni resin.
b) Sonicate the solution on ice using 180 one-second bursts at high intensity with a three-second cooling period.
c) **Optional**: If the lysate is too viscous, add RNase A (10 µg/ml) and DNase I (5 µg/ml) and incubate on ice for 10-15 minutes.
c) Centrifuge the lysate at 10,000 × *g* for 15 minutes to pellet the cellular debris. Apply the supernatant onto the Ni column.

B. For proteins secreted into culture medium by yeast, insect, or mammalian expression systems

a) If the culture supernatant does not contain EDTA, histidine, or any other reducing agents that might affect the Ni column, it can be applied directly to the. Otherwise,
b) Dialyze the sample against 1× PBS before applying it to the column.
d) For large volume of supernatant, concentrate the proteins by ammonium sulphate precipitation, dialyze the dissolved protein solution against 1× PBS, and then apply the solution onto the Ni column.

(2). Column preparation

a) Mix the slurry by gently inverting the bottle several times to completely suspend the resin.
b) Use a pipette to transfer an appropriate volume of Ni resin slurry to the column. Allow the resin to settle and the storage buffer to drain from the column.
c) Equilibrate the column with four bed volumes of LE buffer or until A_{280} is stable.

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(3). Binding the protein to the resin
Apply the cleared sample containing His-tagged protein to the column with a flow-rate of 0.5-1 ml per minute. Collect and save the flow-through for analysis.

(4). Washing

Wash the column with eight bed volumes of Wash buffer or until A_{280} is stable at the flow-rate of 1 ml per minute.

(5). Elution of the target protein

Elute the polyhistidine-tagged protein with five to ten bed volumes of Elution buffer. Collect the elute and dialyze it against 20 mM Tris-HCl pH 8.0 or 1×PBS, pH 7.4 according to the specific application of the target protein.

Example of using this product and comparison with the commercialized Ni-NTA Resin

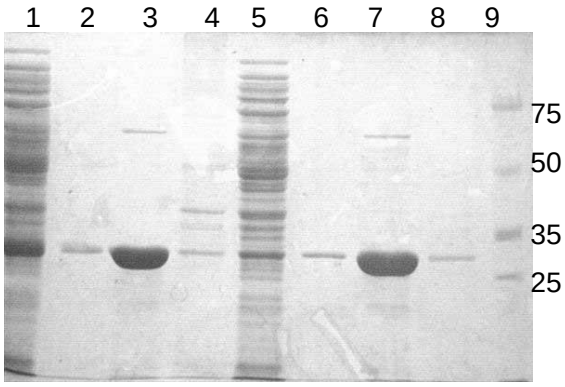

Fig. 1. Comparison of GenScript High Affinity Ni-NTA Resin with that of X Company.
A soluble 30 kD recombinant His-tagged protein was purified from *E. coli* Cell lysate using Ni-NTA Resin from X Company (Lane 1, 2, 3 and 4) and GenScript High Affinity Ni-NTA Resin (Lane 5, 6, 7 and 8), respectively.

1. Flow-through X Company
2. Wash X Company
3. Elute X Company
4. Remainder on resion X Company
5. Flow-through GenScript
6. Wash GenScript
7. Elute GenScript
8. Remainder on resion GenScript

2. Purification of polyhistidine-tagged proteins from *E. coli* under denaturing conditions

This protocol is for target proteins that are expressed mainly in inclusion bodies.

Before use, prepare the following three solutions:

Buffer B	Wash Buffer C	Elution Buffer E
• 100 mM NaH_2PO_4	• 100 mM NaH_2PO_4	• 100 mM NaH_2PO_4
• 10 mM Tris•Cl	• 10 mM Tris•Cl	• 10 mM Tris•Cl
• 8 M urea	• 8 M urea	• 8 M urea
• Adjust pH to **8.0** using 1 M NaOH	• Adjust pH to **6.3** using 1 M HCl	• Adjust pH to **4.5** using 1 M HCl

(1). Resuspend the cell pellet in 1× PBS (about 7.5 ml per ml of pellet), and disrupt cells by sonication as described above.
(2). Collect inclusion bodies by centrifuging the lysate at 12,000 rpm for 10 minutes. Wash inclusion bodies with 1× PBS several times if necessary.
(3). Solubilize the inclusion bodies in Buffer B (about 7.5 ml per ml of pellet), and incubate for 30-60 minutes at room temperature. Homogenization or sonication may be necessary to fully solubilize the pellet.
(4). Centrifuge at 12,000 rpm for 30 minutes to remove any remaining insoluble material. Carefully transfer supernatant to a clean tube without disturbing the pellet and load it on the Ni column pre-equilibrated with Buffer B.
(5). Wash the column with Buffer B until the absorption at 280 nm is close to zero.
(6). Wash the column with two bed volumes of Buffer C. (Note: This buffer is more stringent than Buffer B.)
(7). Elute with minimal volume of Buffer E.

Note: The process recommended here is the purification of protein from inclusion body, the eluted protein from this process may need to be refolded to obtain the active and soluble protein.

IV. REGENERATION OF THE RESIN

For complete regeneration, wash the resin with the following solutions:

1. 2 bed volumes of 6 M GuHCl, 0.2 M acetic acid
2. 5 bed volumes of deionized water
3. 3 bed volumes of 2% SDS
4. 5 bed volumes of deionized water
5. 5 bed volumes of 100% EtOH
6. 5 bed volumes of deionized water
7. 5 bed volumes of 100 mM EDTA (pH 8)
8. 5 bed volumes of deionized water
9. 5 bed volumes of 100 mM $NiSO_4$
10. 10 bed volumes of deionized water

V. TROUBLESHOOTING

Problem	Possible Cause	Solution
No recombinant protein is recovered following elution.	The His-tag is not exposed because of protein folding.	Try denaturing conditions.
	The expression level is too low.	Optimize the expression conditions.
	Not enough sample is loaded.	Load more sample.
	The protein is eluted by too much stringent washing.	Do not use wash Buffer C.
	The recombinant protein has very high affinity for the resin.	Increase the stringency of the elution by decreasing the pH or increasing the imidazole concentration. Use EDTA or EGTA (10-100 mM) to strip the resin of nickel ions and elute the protein.
	The protein is degraded.	Perform all purification steps at 4°C and use protease inhibitors.
The recombinant protein recovered is not pure.	The Resin is not washed well.	Wash with more bed volumes of Buffer C.
	There are other His-rich proteins in sample.	Try an additional wash with a high-stringency buffer of lower pH (between pH 4 and pH 6) before the elution step. Try a pH gradient elution or an imidazole gradient elution. Perform a second purification over another type of column.
The column turns white.	Chelating agents present in the buffer strip the nickel ions from the column.	Recharge the column with Ni as described on page 3.

VI. ORDERING INFORMATION

High Affinity Ni-NTA Resin: Cat. No. L00250

For Research Use Only.

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