High Affinity Ni-Charged Resin Cat. No. L00223

Technical Manual No. TM0217 **Version 11142013**

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1. Product Description

GenScript High Affinity Ni-Charged Resin is an 4% cross-linked agarose medium covalently coupled to a chelating agent that binds Ni^{2+} by four coordination sites for high-affinity purification of polyhistidine-tagged recombinant proteins. High Affinity Ni-Charged Resin has low Ni^{2+} leakage, high protein-binding capacity and stability, and is compatible with a wide range of additives used in protein purification. This makes High Affinity Ni-Charged Resin the excellent choice for high performance purification of polyhistidine-tagged proteins. High Affinity Ni-Charged Resin is available in 10, 25 and 500 ml lab packs.

Table 1. Characteristics of High Affinity Ni-Charged Resin

Resin Volume	10, 25 and 500 ml settled resin (20, 50 and 1000 ml 50% slurry)
Dynamic binding capacity	20 mg of 6xHis-tagged protein (27 kDa) /ml settled resin
Matrix spherical	4% cross-linked agarose
Average particle size	90 µm (45-165 µm)
Storage solution	1X PBS containing 20% ethanol
Storage & Stability	18 months when stored unopened at 2-8 °C

Table 2. Reagents Compatible with High Affinity Ni-Charged Resin

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

2. Purification Procedure

2.1 Purification of polyhistidine-tagged proteins under native conditions

Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.45 µm filter before use.

Lysis Equilibration Buffer (LE buffer): 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0

Wash Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0

Elution buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0

Sample Preparation

1. For protein expressed in *E. coli* or yeast cytoplasm.
   1) Harvest cells from a 50 ml culture by centrifugation at 4 ℃ (*e.g.*, 5,000 rpm for five minutes in a Sorvall SS-34 rotor).
   2) Resuspend the cells in 8 ml of LE buffer with appropriate amount of PMSF or other protease inhibitors added. **Note:** The inhibitors must have no effect on the ability of the Ni^{2+} resin.
   3) Sonicate the solution on ice using 180 one-second bursts at high intensity with a three-second cooling period. **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.
   4) Centrifuge the lysate at 12,000 rpm for 15 minutes at 4 ℃ to pellet the cellular debris. Apply the supernatant onto the Ni^{2+} column.
2. For proteins secreted into culture medium by yeast, insect, or mammalian expression systems.
   1) If the culture supernatant does not contain EDTA, histidine, or any other reducing agents that might affect the Ni^{2+} column, it can be applied directly to the column. Otherwise, perform the following procedures.
   2) Dialyze the sample against 1 × PBS before applying it onto the column.
   3) 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^{2+} column.

Column Preparation

1. Mix the slurry by gently inverting the bottle several times to completely suspend the resin.
2. Transfer an appropriate volume of the slurry to the column. Allow the resin to settle down and the storage buffer to drain from the column.
3. Equilibrate the column with 4 × bed volumes of LE buffer or until A_{280} is stable.

Column Purification

1. Apply the clear sample containing target polyhistidine-tagged protein onto the column with a flow-rate of 0.5 - 1 ml per minute. Collect and save the flow-through for analysis.
2. Wash the column with 8 × bed volumes of Wash buffer or until A_{280} is stable at the flow-rate of 1 ml per minute.
3. Elute the polyhistidine-tagged protein with 5 to 10 × bed volumes of Elution Buffer at the flow-rate of 0.5 - 1 ml per minute. 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.

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

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

Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.45 μm filter before use.

Lysis Equilibration Buffer (LE buffer): 100 mM NaH_2PO_4, 10 mM Tris•Cl, 8 M urea, pH 8.0

Wash Buffer: 100 mM NaH_2PO_4, 10 mM Tris•Cl, 10 mM Imidazole, 8 M urea, pH 8.0

Elution buffer: 100 mM NaH_2PO_4, 10 mM Tris•Cl, 250 mM Imidazole, 8 M urea, pH 8.0

Solubilization of Inclusion Bodies

1. Resuspend the cell pellet in cold (4℃) 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 at 4℃. Wash inclusion bodies with 1 × PBS several times if necessary.
3. Solubilize the inclusion bodies in LE buffer (about 7.5 ml/ml 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.

Column Purification

1. Carefully transfer supernatant to a clean tube without disturbing the pellet and load it onto the Ni^{2+} column pre-equilibrated with LE Buffer.
2. Wash the column with LE Buffer until the absorption at 280 nm is close to zero.
3. Wash the column with 2 × bed volumes of Wash Buffer.
4. Elute with minimal volume of Elution Buffer.

Note: The protocol recommended here is to purify target protein from inclusion bodies, thus the eluted protein from this process may need to be refolded to obtain the active and soluble protein.

Regeneration of Column

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
11. For long-term storage, the resin should be stored in 1×PBS containing 20% ethanol at 2 - 8°C.

3. Troubleshooting

Problem	Possible Cause	Solution
The yield of the purified polyhistidine-tagged protein is low or undetectable.	The polyhistidine tag is not exposed because of protein folding.	Try denaturing conditions.
	The expression level is too low.	Optimize the expression conditions.
	Not enough samples are loaded.	Load more sample.
	The protein is eluted by too much stringent washing.	Use LE Buffer instead of Wash Buffer to wash the resin.
	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.
Multiple bands observed in the eluted protein.	The resin is not washed well.	Wash with more bed volumes of Wash Buffer.
	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 resin
The column turns white.	Chelating agents present in the buffer strip the nickel ions from the column.	Recharge the column with Ni^{2+} as described on page 4.

4. Ordering Information

High Affinity Ni-Charged Resin: Cat. No. L00223-10

L00223-25

L00223-500

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