Version：03
Update：03/01/2024

High Affinity Ni-Charged Resin FF
Cat No: L00666

I. Product Description....................1
II. Purification Procedure.................... 3
III. Cleaning in place....................5
IV. Regeneration of Column.................... 5
V. Troubleshooting....................6
VI. Ordering Information....................6
VII. References....................7

I. Product Description

GenScript **High Affinity Ni-Charged Resin FF** is an 6% highly 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 (see Figure 1) [1]. **High Affinity Ni-Charged Resin FF** 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 FF** the excellent choice for high performance purification of polyhistidine-tagged proteins.

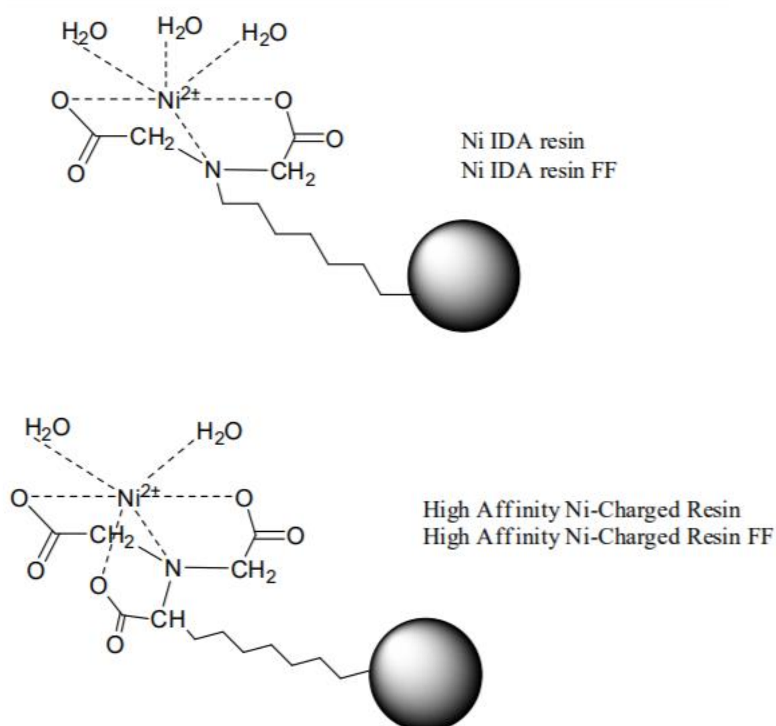

Figure 1. Schematic diagram of the chemical structure of Ni IDA Resin, Ni IDA Resin FF, Hi affinity Ni-Charged Resin and Hi affinity Ni-Charged Resin FF

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

Table 1. Characteristics of High Affinity Ni-Charged Resin FF

Matrix spherical	6% highly cross-linked agarose
Average particle size	90 µm (45-165 µm)
Dynamic Binding capacity[1]	≥50 mg of histidine-tagged protein /mL settled resin
Storage solution	20% ethanol
Storage temperature	stored at 2-8 °C; DO NOT FREEZE

[1] Dynamic binding capacity conditions:

Sample: 5 mg/mL (histidine)$_6$-tagged pure proteins (M_r 43 000) in binding buffer (capacity at 10% Breakthrough)

Column volume: 1 mL

Retention time: 3min

Flow rate: 0.333 mL/min

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 250 mM imidazole, pH 7.4

Note: Dynamic binding capacity is protein-dependent.

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

Reducing agents	1 mM DTT
	20 mM β-ME
	5mM TCEP-HCl
	10 mM reduced glutathione
Denaturants	6 M Gua·HCl†
	8 M Urea†
Detergents	2% Triton X-100
	2% Tween 20
	50% glycerin
Salts	4 M $MgCl_2$
	5 mM $CaCl_2$
	2 M NaCl
	100mM Na_2SO_4
others	50% glycerol
	20% ethanol
	1 mM EDTA‡
	60 mM citrate†

† Tested for 1 week at 40°C.

‡ The strong chelator EDTA has been used successfully in some cases at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not in buffers). Any metal ion stripping may be counteracted by addition of a small excess of MgCl2 before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

II. 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.

Table 3. Buffer and formula required for purification of polyhistidine-tagged proteins under native conditions

Name	Volume	Formula
Lysis Equilibration Buffer (LE buffer)	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \cdot 2H_2O$) 300 mM NaCl (17.54 g NaCl) Adjust pH to 8.0 with NaOH
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) 10 mM imidazole (0.68 g imidazole) Adjust pH to 8.0 with NaOH
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 pH to 8.0 with NaOH

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 one-second bursts at high intensity with a three-second cooling period. Total sonication time is about 30 to 45 minutes.

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 A280 is stable.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

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 A280 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.

Table 4. Buffer and formula required for purification from *E. coli* under denaturing conditions

Name	Volume	Formula
Lysis Equilibration Buffer (LE buffer)	1 L	100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 10 mM Tris•Cl (1.21 g Tris, add 980mL dd H_2O, Adjust pH to 8.0 with HCl, Volume to 1000 mL.) 8 M Urea (480.50 g urea) Adjust pH to 8.0 with NaOH
Wash Buffer	1 L	100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 10 mM Tris•Cl (1.21 g Tris, add 980mL dd H_2O, Adjust pH to 8.0 with HCl, Volume to 1000mL.) 8 M Urea (480.50 g urea) 10 mM imidazole (0.68 g imidazole) Adjust pH to 8.0 with NaOH
Elution buffer	1 L	100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 10 mM Tris•Cl (1.21 g Tris, add 980mL dd H_2O, Adjust pH to 8.0 with HCl, Volume to 1000mL.) 8 M Urea (480.50 g urea) 250 mM imidazole (17.0 g imidazole) Adjust pH to 8.0 with NaOH

Solubilization of Inclusion Bodies

1. Resuspend the cell pellet in cold (4℃) 1 × PBS (about 7.5 mL PBS per g 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/g 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 preequilibrated with LE Buffer.

2. Wash the column with LE Buffer until the absorption at 280 nm is close to zero.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

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.

2.3 SDS-PAGE Analysis

After the protein is purified, perform SDS-PAGE analysis. Take an appropriate amount of the samples (including elution components, wash components and elution components) obtained from the purified products and the original samples and mix them with the loading buffer, respectively, then perform gel electrophoresis. After that, complete the staining in a short time with an estain protein stainer.

	model
SDS-PAGE	SurePAGE™
	eStain™ L1 Protein Staining System

Use SDS-PAGE to test the purification effect of the samples (including elution components, wash components and elution components) obtained from the purified products and the original samples.

III. Cleaning in place

When the back pressure is too high or obvious contamination appears on the packing during the use of the packing, cleaning-in-place (CIP) operations are required.

It is recommended to follow the steps below to remove residual contaminants on the filler, such as precipitated proteins, hydrophobins, and lipoproteins.

Remove strong hydrophobic binding proteins, lipoproteins and lipids

This type of contaminants can be removed by washing 5-10 10 × bed volumes with 30% isopropanol and a contact time of 15-20 minutes.

Then, rinse the column with 10 × bed volumes of deionized water. You can also choose to use an acidic or alkaline solution containing detergent to clean the packing by 2 × bed volumes. For example, 0.1 M acetic acid solution containing 0.1–0.5% non-ionic detergent, with a contact time of 1–2 hours. After the detergent is treated, it is necessary to wash 5 × bed column volumes with 70% ethanol to completely remove the detergent.

Finally, Wash the column with 10 × bed volumes of deionized water.

Remove ion-bound proteins

Wash 10-15 minutes with 1.5M NaCl solution. Then, rinse another the column with 10 × bed volumes of deionized water.

IV. Regeneration of Column

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

1. 2 × bed volumes of 6 M GuHCl and 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 $NiSO_4$/$CuSO_4$/$CoCl_2$
10. 10 × bed volumes of deionized water
11. For long-term storage, the resin should be stored in 20% ethanol at 2 - 8°C.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

V. Troubleshooting

Problem	Possible Cause Solution	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 sample is loaded.	Load more sample.
	The protein was 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 was not washed well.	Wash with more bed volumes of Wash Buffer.
		Try a pH gradient elution or an imidazole gradient elution.
	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 2 (Regeneration of Column).

VI. Ordering Information

Product Name	Cat. No.
Ni Resin FF	L00465
High Affinity Ni-Charged Resin FF Prepacked Column	L00683
Ni-IDA Resin FF Prepacked Column	L00684
Ni-charged MagBeads	L00295
AmMag™ Ni Magnetic beads	L00776

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188

VII. References

[1] Rhichard R. Burgess, *etc*. Methods in enzymology. *[J]* USA. Elsevier. 2009, 463.

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.

生产商：南京金斯瑞生物科技有限公司 江苏省南京市江宁区科学园雍熙路 28 号
Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
Tel: 1-877-436-7274; 1-732-885-9188