

Ni Resin FF
Cat. No. L00465-5;L00465-25**Version 11142014**

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I Product Description

GenScript Ni Resin FF is a 6% highly cross-linked agarose medium covalently coupled to a chelating agent that binds Ni²⁺ by three coordination sites for high-affinity purification of polyhistidine-tagged recombinant proteins. Ni Resin FF has low Ni²⁺ leakage, high protein-binding capacity and stability, and is compatible with a wide range of additives used in protein purification. Ni Resin FF is an excellent choice for high performance purification of polyhistidine-tagged proteins.

Table 1. Characteristics of Ni Resin FF

Binding capacity	>40 mg of histidine-tagged protein /ml settled resin
Matrix spherical	6% highly 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

II Purification Procedure

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 that buffers be filtered prior to use by passing them through a 0.45 µm filter.

Lysis Equilibration Buffer (LE buffer): 50 mM NaH₂PO₄, 300 mM NaCl, pH 8.0

Wash Buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0

Elution buffer: 50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole, pH 8.0

Sample Preparation

1. For proteins expressed in *E. coli* or yeast cytoplasm
 - 1) Harvest cells from a 50 ml culture by centrifugation at 4° Celsius (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 the appropriate amount of PMSF, or other protease inhibitors, added. **Note:** Inhibitors that could compromise Ni²⁺ resin's integrity should not be used.
 - 3) Sonicate the solution on ice using one-second bursts at high intensity with a three-second cooling period. Total sonication time is approximately 30 to 45 min.
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° Celsius to pellet the cellular debris. Transfer the supernatant onto the Ni²⁺ 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 Ni²⁺ column, it can be added directly into the column. Otherwise, perform the following procedures.
 - 2) Dialyze the sample against 1 × PBS before adding it into the column.
 - 3) For large volumes of supernatant, concentrate the proteins by ammonium sulphate precipitation, dialyze the dissolved protein solution against 1 × PBS, and then transfer the solution into the Ni²⁺ 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.

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 eluate 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.

III 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.	
		Try a pH gradient elution or an imidazole gradient elution.	
	There are other His-rich proteins in the 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.	

IV Ordering Information

Name	Cat. No.	Size
Ni Resin FF	L00465-5	5 ml
Ni Resin FF	L00465-25	25 ml

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The logo for witec ag features a blue circle above the letter 'i' in 'witec', which is written in a bold, white, sans-serif font. The letters 'ag' are also in a bold, white, sans-serif font and are positioned to the right of 'witec'.

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