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# NEBExpress Ni Resin Batch Binding Typical Protocol (NEB #S1428) V.1

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

NEBExpress® Ni Resin is an affinity matrix for the isolation and purification of polyhistidine-tagged (His-tagged) *fusion proteins*. It is intended for use in gravity *or* pressure flow columns, *and* batch purifications. NEBExpress Ni Resin is comprised of a highly uniform and stable chemical-tolerant resin, pre-charged with nickel ions on the matrix surface. It is resistant to a wide range of chemicals, including NaOH, EDTA, DTT and  $\beta$ -Mercaptoethanol.

- Purification of  $\geq 10$  mg His-tagged protein per 1 ml of resin
- Intended for use in gravity or pressure flow columns, and batch purifications
- High specific binding of His-tagged proteins yielding purities of  $>95\%$
- Strong nickel ion binding provides excellent resistance to EDTA and reducing agents. Compatible with commercially available detergent-based cell lysis reagents
- Isolation and purification of His-tagged fusion proteins under native or denaturing conditions



## Guidelines

### Chemical Compatibility

Reagent	Tolerance (up to)
EDTA	10 mM*, 100 mM*
DTT	5 mM
b-mercaptoethanol	20 mM
TCEP	5 mM
Triton X-100	2 %
Tween 20	2 %
NP-40	2 %
Cholate	2 %
CHAPS	1 %
Tris-HCl, HEPES, MOPS	100 mM
Urea	8 M
Guandine-HCl	6 M

\* If reagents contain 10 mM EDTA, do not mix the sample and the resin for more than 24 hours before washing and eluting.

\*\* If reagents contain 100 mM EDTA, do not mix the sample and the resin for more than 2 hours before washing and eluting.



## Materials

### MATERIALS

 NEBExpressNi Resin – 25 ml **New England Biolabs Catalog #S1428S**


#### **Recommended Buffers**

**Lysis/Binding Buffer:** 20 mM sodium phosphate, 300 mM NaCl, pH 7.4

**Wash Buffer:** 20 mM sodium phosphate, 300 mM NaCl, 5 mM Imidazole, pH 7.4

**Elution Buffer:** 20 mM sodium phosphate, 300 mM NaCl, 500mM Imidazole, pH 7.4

## Safety warnings

 Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

## Before start

*Ni resin can be used for the purification of His-tagged fusion proteins under native or denaturing conditions*

·The binding capacity of NEBExpress<sup>®</sup> Ni Resin is  $\geq 10$  mg/ml. The binding capacity will vary depending on the size of the target protein, binding conditions and the accessibility of the His-tag. An exact protocol may need to be optimized by the user.

·It is recommended to estimate the expression level of the His-tagged protein of interest by first running a sample on an SDS-PAGE gel.

Purchase or prepare recommended buffers:

#### **Recommended Buffers**

**Lysis/Binding Buffer:** 20 mM sodium phosphate, 300 mM NaCl, pH 7.4

**Wash Buffer:** 20 mM sodium phosphate, 300 mM NaCl, 5 mM Imidazole, pH 7.4

**Elution Buffer:** 20 mM sodium phosphate, 300 mM NaCl, 500mM Imidazole, pH 7.4

## Preparation of Buffers under Denaturing Conditions

- 1 If continuing with **Denaturing** conditions: Bring all three buffers (Lysis/Binding, Wash and Elution Buffers) to a final concentration of [M] 8 Molarity (M) Urea or [M] 6 Molarity (M) Guanidine .



### Note

#### Notes:

1. Crude lysate should be prepared with a lysis buffer without imidazole. To further minimize contaminants in the eluate, the concentration of imidazole in the wash buffer can be increased to 15 mM ( $\geq 15$  mM can reduce the isolated yield but may result in increased purity). Imidazole concentration should be determined empirically.



2. Refer to the Chemical Compatibility table prior to including other additives.

## Sample Preparation

- 2 Harvest cells by centrifugation at  4000 x g, 00:15:00 , store the pellet at  -20 °C or process immediately.

### Note



Note: it is recommended to pre-weigh the vessel prior to addition of cell suspension, in order to determine the mass of cell pellet used.

- 3 Resuspend cell pellet in Lysis buffer and lyse using method of choice (use approximately  5 mL lysis buffer per  1 g cell paste ).












### Note

Note: Cells can be lysed by standard methods including sonication, repeated freeze-thaw cycles, French press, etc. Other commercially available lysis reagents can also be used, following manufacturer's instructions. It is recommended that imidazole be omitted from any lysis buffer.





- 4 Centrifuge sample at  12000 x g, 00:15:00 to pellet cellular debris. Remove the clarified protein lysate supernatant and transfer to a new microcentrifuge tube on ice, **retain an aliquot of the clarified lysate** for SDS-PAGE analysis. 

## Resin Preparation


- 5 Gently shake the Ni Resin bottle to completely resuspend the slurry and transfer desired amount to a tube. 
- 6 Settle the resin by centrifugation at  500 x g, 00:05:00 , remove the supernatant. 
- 7 Equilibrate the resin by adding 5 column volumes (CV) of Lysis/Binding buffer, shake gently to mix. 
- 8 Settle the resin by centrifugation at  500 x g, 00:05:00 , remove the supernatant. 
- 9 Repeat equilibration steps once:
- 9.1 Settle the resin by centrifugation at  500 x g, 00:05:00 , remove the supernatant. 
- 9.2 Equilibrate the resin by adding 5 column volumes (CV) of Lysis/Binding buffer, shake gently to mix. 
- 9.3 Settle the resin by centrifugation at  500 x g, 00:05:00 , remove the supernatant. 


## Batch Binding

- 10 Add the protein sample lysate to the equilibrated resin. Mix gently by shaking at 200 rpm on a rotary shaker for ≥  00:15:00 . 





#### Note

Note: Binding of some His-tagged proteins can be increased with longer incubation times. Resin should be placed at  4 °C when extending the binding time to maintain activity of the target protein. Prolonged incubation may result in more non-specific binding.

- 11 Pour the lysate/resin mixture into an empty and closed gravity column.
- 12 Open the column and collect the flow through in a clean tube. Reserve for SDS-PAGE analysis. 

### Column Wash


- 13 Add  $\geq 10$  CV of Wash buffer to the column, allow buffer to flow through. Flow through can be monitored with absorbance at 280 nm to ensure complete removal of unbound lysate effluent. 
- 14 Collect flow through in fractions and reserve for SDS-PAGE analysis. 

### Protein Elution

- 15 Elute fusion protein with Elution buffer.

#### Note

Note: it is recommended to use a minimum of 3 CV of elution buffer to elute the target protein. Optimal amounts may need to be determined empirically for different substrates.

- 16 Collect flow through in fractions and monitor protein elution by measuring the absorbance at 280 nm or by SDS-PAGE.
- 17 Analyze the protein sample lysate (load), flow through, washes and eluates by SDS-PAGE. 



### Note

Note: To remove imidazole for downstream applications use gel filtration (e.g., Zeba Spin Desalting Columns) or dialysis (e.g. slide-A-Lyzer Dialysis Cassettes)

## Guidelines for Ni Resin Cleaning

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### Note

When using a gravity column, the flow rate should be determined prior to cleaning to ensure an accurate contact time with the cleaning agent.

To remove lipoproteins, lipids and proteins strongly bound by hydrophobic interaction follow **one** of these three methods:

1. Add [M] 1 Molarity (M) NaOH to the column and allow a contact time of 01:00:00 to 02:00:00 , followed by 10 CV of Lysis/Binding Buffer.
2. Add 30% isopropanol to the column and allow a contact time of 00:15:00 to 00:20:00 , followed by 10 CV of water.
3. Add 0.1 – 0.5% non-ionic detergent in [M] 0.1 Molarity (M) acetic acid to the column and allow a contact time of 01:00:00 to 02:00:00 , followed by 10 CV of water.

19 To remove proteins bound by ionic interactions



1. Add [M] 1.5 Molarity (M) NaCl to the column and allow a contact time of 00:10:00 to 00:15:00 . followed by 10 CV of water.

## Storage

20 For long-term storage, NEBExpress Ni Resin should be stored in 20% ethanol at 2 °C to 8 °C .