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NEBExpress Ni Resin Pressurized Column Typical Protocol (NEB #S1428)

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1 Works for me dx.doi.org/10.17504/protocols.io.bfa4jigw

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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 β-Mercaptoethanol.

- Purification of ≥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

EXTERNAL LINK

https://www.neb.com/protocols/2019/09/10/nebexpress-ni-resin-pressurized-column-protocol-neb-s1428

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
Guanidine-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

NAME	CATALOG #	VENDOR
NEBExpressNi Resin – 25 ml	S1428S	New England Biolabs

SAFETY WARNINGS

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

BEFORE STARTING

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BEFORE STARTING

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

·The binding capacity of NEBExpress® Ni Resin is ≥ 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 8M Urea or 6M Guanidine.

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 (≥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: 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 of lysis buffer per gram of cell paste).

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.

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Pressurized Column Purification

5

NEBExpress™ Ni Resin is suitable for large scale purifications using a pressurized FPLC system.

Columns should be packed at a constant pressure of approximately 1 bar (0.1 MPa) or a packing flow velocity of approximately 400 cm/h (10cm bed height, 25°C, low viscosity buffer).

6 When running the column purification, the maximum pressure for the resin is 3 bar (0.3 MPa).

Guidelines for Resin Cleaning

7

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 1 M NaOH to the column and allow a contact time of 1 to 2 hours, followed by 10 CV of Lysis/Binding Buffer.
2. Add 30% isopropanol to the column and allow a contact time of 15-20 minutes, followed by 10 CV of water.
3. Add 0.1 – 0.5% non-ionic detergent in 0.1 M acetic acid to the column and allow a contact time of 1 – 2 hours, followed by 10 CV of water.

8 To remove proteins bound by ionic interactions

1. Add 1.5 M NaCl to the column and allow a contact time of 10 – 15 minutes. followed by 10 CV of water.

Storage

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

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