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NEBExpress Ni Spin Column Reaction Protocol (NEB #S1427)

New England Biolabs[1]

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ABSTRACT

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

- The binding capacity of NEBExpress® Ni Spin Columns is ≥ 1 mg per column. The binding capacity can 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.

EXTERNAL LINK

https://www.neb.com/protocols/2019/08/28/nebexpress-ni-spin-column-reaction-protocol-neb-s1427

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 Spin Columns – 25 columns	S1427L	New England Biolabs
NEBExpressNi Spin Columns – 10 columns	S1427S	New England Biolabs

MATERIALS TEXT

Sodium Phosphate
NaCl
Imidazole, pH 7.4
H_2O
Centrifuge
2ml microcentrifuge tubes

SAFETY WARNINGS

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

BEFORE STARTING

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Buffer Preparation for Ni Spin Columns

1 Prepare buffers for Ni Spin Columns as follows:

	Lysis/Binding Buffer: **20 mM sodium phosphate, 300 mM NaCl**	**Wash Buffer:** **20 mM sodium phosphate, 300 mM NaCl, 5 mM Imidazole**	**Elution Buffer:** **20 mM sodium phosphate, 300 mM NaCl, 500 mM Imidazole**
2X IMAC Buffer	7.5 ml	5.0 ml	2.5 ml
2M Imidazole	-	0.025 ml	1.25 ml
H2O	7.5 ml	5 ml	1.25 ml
Total	**15.0 ml**	**10.0 ml**	**5.0 ml**

Notes:
1. When the recommended protocol is followed, each isolation requires the following volumes of buffer: **0.75 ml Lysis/Binding Buffer** , **0.75 ml Wash Buffer** and **0.4 ml Elution Buffer** . An excess volume of each concentrated buffer is provided for preparation of cell lysates or to optimize the concentration of imidazole in the washes.
2. 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 10 mM (≥10 mM can reduce the isolated yield but may result in increased purity). imidazole concentration should be determined empirically.
3. Refer to the Chemical Compatibility table prior to including other additives.

2 To prepare buffers for Ni Spin Columns under **Native** Conditions: bring all three buffers to a final pH of 7.4
To prepare buffers for Ni Spin Columns under **Denaturing** Conditions: bring all three buffers to a final concentration of **8 Molarity (M) Urea** or **6 Molarity (M) Guanidine** .

Sample Preparation

3 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.

4 Resuspend cell pellet in Lysis Buffer and lyse using method of choice (use approximately **5 ml lysis buffer** per **1 g 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.

5 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 a 2 µl aliquot of the clarified lysate** for SDS-PAGE analysis.

Note: A standard isolation typically employs 0.5 ml of clarified lysate.

Column Preparation

6 Remove the bottom tab of the column by twisting, loosen the top cap and place the column in the collection tube provided.

7 Centrifuge column at **800 x g 00:01:00** to remove the storage buffer, discard the buffer.

8 Add **250 µl Lysis/Binding buffer** to the column.

9 Centrifuge column at **800 x g 00:01:00** , discard the Lysis/Binding buffer.

10 Place the column in a new 2 ml microcentrifuge tube.

Lysate Binding

11 Add **up to 500 µl protein sample lysate** to the column.

Note: If sample volume is greater than 500 µl multiple applications can be performed; collect the flow through in separate microcentrifuge tubes.

12 Tap the column to mix the lysate with the resin and allow binding for **00:02:00** .

Note: Binding of some His-tagged proteins can be increased with longer mixing times. Cap the column and seal the bottom using the plug provided. Mix end-over-end at **4 °C** for desired time (typically **00:05:00** - **00:15:00**). Prolonged mixing may result in more non-specific binding.

13 Centrifuge column at **800 x g 00:01:00** , reserve flow through.

14 Place the column in a new 2 ml microcentrifuge tube.

Column Wash

15 Add **250 µl wash buffer** to the column and centrifuge at **800 x g 00:01:00** .

16 Repeat wash step twice, collect each wash in a separate 2 ml microcentrifuge tube: Add **250 µl wash buffer** to the column and centrifuge at **800 x g 00:01:00** . (1/2)

17 Repeat wash step twice, collect each wash in a separate 2 ml microcentrifuge tube: Add **250 µl wash buffer** to the column and centrifuge at **800 x g 00:01:00** . (2/2)

Protein Elution

18 Place column in a new 2 ml microcentrifuge tube.

19 Add **200 µl elution buffer** to the column. Mix the resin with the elution buffer thoroughly.

Note: Elution volume can be reduced to **100 µl** if a more concentrated protein sample is desired, total target protein yield may be lower.

20 Centrifuge at **800 x g 00:01:00** , save eluted sample.

21 Place column in a new 2 ml microcentrifuge tube and repeat elution step. **Typically, >90% of the bound protein is eluted following the second elution.**

22 Analyze the clarified cell lysate (load), flow through, washes and eluates by SDS-PAGE.