NEBExpress® Ni Spin Column Reaction Protocol (NEB #S1427)

Overview

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.

Buffer Preparation for Ni Spin Columns

Supplied Concentrated Buffers:

B1076, 2X IMAC Buffer (0.04 M Sodium Phosphate, 0.6 M NaCl, pH 7.4)

B1077, 2M Imidazole (2M Imidazole, pH 7.4)

To prepare buffers for Ni Spin Columns under Native Conditions

	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,** **500 mM Imidazole, pH 7.4**
2X IMAC Buffer	7.5 ml	5.0 ml	2.5 ml
2M Imidazole	-	0.025 ml	1.25 ml
H_2O	7.5 ml	5 ml	1.25 ml
Total	**15.0 ml**	**10.0 ml**	**5.0 ml**

Preparation of Ni Spin Columns under Denaturing Conditions

1. Bring all three buffers (Lysis/Binding, Wash and Elution Buffers) to a final concentration of 8M Urea or 6M Guanidine.

Notes:

1. *When the recommended protocol is followed, each isolation requires the following volumes of buffer: 0.75 ml of Lysis/Binding Buffer, 0.75 ml of Wash Buffer and 0.4 ml of 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.*

Sample Preparation

1. Harvest cells by centrifugation at 4,000 x g for 15 minutes, 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.

2. 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.

3. Centrifuge sample at 12,000 x g for 15 minutes 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

1. Remove the bottom tab of the column by twisting, loosen the top cap and place the column in the collection tube provided.
2. Centrifuge column at 800 x g for 1 minute to remove the storage buffer, discard the buffer.
3. Add 250 µl of Lysis/Binding buffer to the column.
4. Centrifuge column at 800 x g for 1 minute, discard the Lysis/Binding buffer.
5. Place the column in a new 2 ml microcentrifuge tube

Lysate Binding

1. Add up to 500 µl of the 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.

2. Tap the column to mix the lysate with the resin and allow binding for 2 minutes.

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 5-15 minutes). Prolonged mixing may result in more non-specific binding.

3. Centrifuge column at 800 x g for 1 minute, reserve flow through.
4. Place the column in a new 2 ml microcentrifuge tube.

Column Wash

1. Add 250 µl of Wash Buffer to the column and centrifuge at 800 x g for 1 minute.
2. Repeat wash step twice, collect each wash in a separate 2 ml microcentrifuge tube.

Protein Elution

1. Place column in a new 2 ml microcentrifuge tube.
2. Add 200 µl of 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.

3. Centrifuge at 800 x g for 1 minute, save eluted sample.
4. 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.
5. Analyze the clarified cell lysate (load), flow through, washes and eluates by SDS-PAGE.

Chemical Compatibility

Reagent	Tolerance (up to)
EDTA	10 mM[1], 100 mM[2]
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

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

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