May 29, 2020

NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)

New England Biolabs[1]

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ABSTRACT

NEBExpress® Ni-NTA Magnetic Beads can be used for the purification of His-tagged fusion proteins under native or denaturing conditions

- The binding capacity of NEBExpress Ni-NTA Magnetic Beads can vary depending on target and binding conditions. We recommend estimating approximately 7.5 mg of available binding capacity per ml (bed volume) of resin, therefore 50 µl of Ni-NTA bead slurry yields approximately 40 µg of purified protein. An exact protocol may need to be optimized by the user. See the FAQs for more information on protocol optimization.

- For optimal performance, the amount of beads used should match the approximate amount of His-tagged protein to be captured.

- Crude lysate should be prepared with lysis buffer or a buffer in the pH range of 7.0–8.2 supplemented with up to 10 mM imidazole to reduce non-specific binding of proteins.

- Magnetic racks may be purchased separately: 6-tube Magnetic Separation Rack (NEB #S1506); 12-tube Magnetic Separation Rack (NEB #S1509); and 96-Well Microtiter Plate Magnetic Separation Rack (NEB #S1511)

EXTERNAL LINK

https://www.neb.com/protocols/2018/11/06/nebexpress-ni-nta-magnetic-beads-typical-reaction-protocol

MATERIALS

NAME	CATALOG #	VENDOR
Ni-NTA Magnetic Beads – 5 ml	S1423L	New England Biolabs

MATERIALS TEXT

Sodium Phosphate
NaCl
H_2O
1.5ml tube
Benchtop shaker
Magentic rack
96-Well Microtiter Plate Magnetic Separation Rack
Incubator
Magnet

SAFETY WARNINGS

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

BEFORE STARTING

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Buffer Preparation for NEBExpress Ni-NTA Magnetic Beads

1 Prepare the following buffers:

	Lysis/Binding Buffer: 20 mM sodium phosphate, 300 mM NaCl, 10 mM Imidazole	**Wash Buffer: 20 mM sodium phosphate, 300 mM NaCl, 20 mM Imidazole**	**Elution Buffer: 20 mM sodium phosphate, 300 mM NaCl, 500 mM Imidazole**
2X IMAC Buffer	1.5 ml	10 ml	1.25 ml
2M Imidazole	0.015 ml	0.2 ml	0.625 ml
H2O	1.49 ml	9.8 ml	0.625 ml
Total	**3.0 ml**	**20 ml**	**2.5 ml**

Notes:
1. Working buffers are sufficient for 10 reactions using the supplied concentrated buffers; volumes can be scaled up for larger reactions

2. Ni-NTA beads have a high affinity for His-tagged proteins with minimum non-specific binding. Under native conditions, the stringency of binding is modulated by including a low concentration of imidazole in the binding and wash buffers. The optimal concentration of imidazole in the buffers may need to be optimized depending on the affinity of the target protein for the Ni-NTA beads.

2 To prepare buffers for NEBExpress Ni-NTA Magnetic Beads under **Native** Conditions: bring all three buffers to a final concentration of pH 7.4

To prepare buffers for NEBExpress Ni-NTA Magnetic Beads under **Denaturing** Conditions: bring all three buffers to a final concentration of [M]**8 Molarity (M) Urea** or [M]**6 Molarity (M) Guanidine** .

3 Move forward with the protocol, using Single Tubes or 96-well Plates:
Step 3 includes a Step case.
Single Tubes
96-well Plates

Bead Equilibration - Single Tubes

step case

Single Tubes

4 Resuspend the bead slurry by vortexing or mixing.

5 Immediately dispense **50 µl bead slurry** to a 1.5 ml tube.

6 Add **200 µl binding buffer** to the bead slurry and mix briefly.

7 Place the tube in a magnetic rack to pellet the beads, remove and discard the supernatant.

Target Protein Binding - Single Tubes

8 Add **1 ml crude lysate** to the equilibrated beads.

9 Incubate for **00:30:00** at **Room temperature** with end over end mixing or with a benchtop shaker at 850 rpm.

Note: Beads may adhere to sides or cap of the tubes during mixing. Samples can be spun briefly in a microcentifuge to pull bead sample down prior to pelleting with magnetic rack.

10 Place tube in a magnetic rack and remove supernatant. Reserve supernatant as flow through.

Wash - Single Tubes

11 Add **500 µl wash buffer** to the bead pellet, mix briefly to re-suspend the beads.

12 Place the tube in a magnetic rack to pellet the beads and remove the supernatant.

13 Repeat wash step twice: Add **500 µl wash buffer** to the bead pellet, mix briefly to re-suspend the beads. (1/2)

14 Place the tube in a magnetic rack to pellet the beads and remove the supernatant. (1/2)

15 Repeat wash step twice: Add **500 µl wash buffer** to the bead pellet, mix briefly to re-suspend the beads. (2/2)

16

Place the tube in a magnetic rack to pellet the beads and remove the supernatant. (2/2)
Remove any remaining wash buffer from the bead pellet and discard in the last wash.

Elution - Single Tubes

17 Add **100 µl elution buffer** and mix the suspension for **00:02:00** on a benchtop shaker at 850 rpm.

18 Place the tube in a magnetic rack to pellet the beads, remove and keep the supernatant containing the eluted target protein.

19 Elution step can be repeated and eluates combined, however the majority of the target protein is in the first elution.