Loading of PureCube NTA or IDA MagBeads with nickel

Overview

This protocol describes the loading of PureCube NTA or IDA MagBeads with nickel solutions, to obtain Ni-NTA or Ni-IDA MagBeads. **Please refer to the appropriate protocol for loading with other transition metals.** T

Amounts given in this protocol are for 1 mL NTA or IDA MagBead suspension, which contains 250 µL magnetic beads. The Cube MagBead Separator holds 1.5 and 2 ml microfuge tubes for convenient separation of MagBeads from the supernatant.
This reaction can be linearly scaled up or down. Magnetic holders for a wide range of volumes are available e.g. from Sepmag (www.sepmag.eu).

Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@ www.cube-biotech.com). Additional protocols can also be found at www.cube-biotech/protocols.

Equipment

- ☐ Magnetic separator for microcentrifuge tubes (e.g. Cube Biotech 16941)
- ☐ Microcentrifuge tubes (2 mL)
- ☐ Vortex mixer

Materials

- ☐ PureCube NTA MagBeads (1 mL, Cube Biotech #31801) or PureCube IDA MagBeads (1 mL, Cube Biotech #30801)
- ☐ Nickel II sulfate
- ☐ Sodium chloride
- ☐ Sodium acetate trihydrate
- ☐ Tris base
- ☐ Acetic acid
- ☐ Ethanol
- ☐ Hydrochloric acid

Solutions and buffers

Sodium acetate buffer, pH 6.0, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Sodium acetate trihydrate	50 mM	136.08	n.a.	680 mg
Instructions: Dissolve sodium acetate in 80 mL water, adjust the pH to 6.0 with acetic acid. Add water to a total volume of 100 mL.				

Nickel sulfate solution, 20 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Nickel II sulfate hexahydrate	2.5%	262.85	n.a.	500 mg
Instructions: Dissolve in 20 ml water.				

Wash Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Sodium chloride	150 mM	58.44	n.a.	877 mg
Acetic acid	200 mM	60.05 Density 1.05 g/mL		1.14 mL
Instructions: Dissolve sodium chloride in 80 mL water, then add acetic acid. Add water to a total volume of 100 mL.				

Tris buffer, pH 7.5, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Tris base	20 mM	121.14		242 mg
Instructions: Dissolve Tris base in 80 mL water, adjust the pH to 7.5 with hydrochloric acid. Add water to a total volume of 100 mL.				

MagBead Storage Buffer, pH 6.5, 250 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Sodium acetate trihydrate	20 mM	136.08	n.a.	135 mg
Ethanol	20 % (v/v)		100 % (v/v)	10.2 mL
Instructions: Dissolve sodium acetate in 30 mL water, adjust the pH to 6.5 with acetic acid. Add 9.6 mL water and 10.2 mL ethanol to yield a total volume of 50 mL.				

Procedure

1. Transfer 1 mL PureCube NTA or IDA MagBeads into a 2 mL microcentrifuge tube.
2. Place the tube on a magnetic stand and allow the beads to separate. Remove the supernatant. Resuspend the magnetic beads with 1 mL double distilled water.
3. Separate the beads and wash two more times with water.
4. Wash 3x with 50 mM sodium acetate, pH 6.0.
5. Wash 1x with double distilled water.
6. Add 1 ml 2.5% nickel sulfate solution and incubate for 2 h on an end-over-end shaker.
7. Wash 4x with double distilled water.
8. Add 1 mL Wash Buffer and incubate for 10 min.
9. Wash 1x with double distilled water.
10. Wash 6x with 20 mM Tris-HCl, pH 7.5.
11. Wash 1x with double distilled water.
12. Resuspend the Ni-NTA or Ni-IDA MagBeads in 1 mL MagBead Storage buffer, yielding a 25% suspension. Store at 4°C.

Tip: The loading reaction can be scaled up and down linearly, by increasing or decreasing the amounts of buffers and solutions described in this protocol.