Loading of PureCube NTA or IDA Agarose with nickel

Overview

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

Amounts given in this protocol are for 20 mL NTA or IDA of 50% Agarose suspension, which contains 10 mL agarose. The reaction can be linearly scaled up and down as required.

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

- ☐ Centrifuge for 50 mL tubes
- ☐ Centrifuge tubes, 50 mL (e.g. Falcon)
- ☐ Vortex mixer
- ☐ End-over-end shaker

Materials

- ☐ PureCube NTA Agarose (10 mL, Cube Biotech #31703) or PureCube IDA Agarose (10 mL, Cube Biotech #30703)
- ☐ 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, 50 mL

Component	Final concentration			Amount needed for buffer
Nickel II sulfate hexahydrate	2.5% (w/v)			1.25 g
Instructions: Dissolve in 50 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, 200 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for buffer
Tris base	20 mM	121.14		484 mg
Instructions: Dissolve Tris base in 160 mL water, adjust the pH to 7.5 with hydrochloric acid. Add water to a total volume of 200 mL.				

Agarose Storage Buffer, pH 6.5, 50 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 20 mL PureCube NTA or IDA Agarose suspension into a 50 mL centrifuge tube.
2. Spin the tube for 5 min at 500 x g to pellet the agarose. Remove the supernatant. Resuspend with 20 mL double distilled water.
3. Wash two more times with 20 mL water.
4. Wash 3x with 20 mL 50 mM sodium acetate, pH 6.0.
5. Wash 1x with 20 mL double distilled water.
6. Add 20 ml 2.5% nickel sulfate solution and incubate for 2 h.
7. Wash 4x with 20 mL double distilled water.
8. Add 20 mL Wash Buffer and incubate for 10 min.
9. Wash 1x with 20 mL double distilled water.
10. Wash 6x with 20 mL 20 mM Tris-HCl, pH 7.5.
11. Wash 1x with 20 mL double distilled water.
12. Resuspend the Ni-NTA or Ni-IDA Agarose in 20 mL Agarose Storage buffer, yielding a 50% 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.