Washing and Regenerating Ni-NTA and Ni-IDA Agarose

Overview

NTA and IDA agarose resins should be washed after each run, and regenerated latest after 5 runs (though we recommend to regenerate the resin after each run, if possible). This protocol delineates washing and regenerating procedures for PureCube Ni-NTA, PureCube 100 Ni-NTA and PureCube Ni-IDA Agarose, including a specific procedure for resins that have been exposed to reducing agents such as DTT. Volumes are given in *column bed volume* (bv), i.e., 10 bv calls for 10 mL of buffer for a 1 mL column bed volume. This protocol can also be implemented for NTA and IDA resins loaded with other metals (e.g., Co, Fe, Al, Cu), using the appropriate solutions to recharge the resin. **The novel INDIGO ligand cannot be stripped with EDTA.** Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@cube-biotech.com); other protocols can also be found at www.cube-biotech/protocols.

Equipment

- ☐ Disposable gravity flow columns with capped bottom outlet, 2 ml, (e.g. Pierce / ThermoScientific #29920)

Materials

- ☐ Sodium hydroxide (NaOH)
- ☐ Sodium chloride (NaCl)
- ☐ Ethylenediaminetetraacetic acid (EDTA)
- ☐ N,N-Dimethyldodecylamine-N-oxide (LDAO, 1 g; Cube Biotech #16005)
- ☐ 20% (v/v) Ethanol (C_2H_6O)
- ☐ Nickel sulfate ($NiSO_4$)
- ☐ Hydrochloric acid (HCl)
- ☐ Acetic acid
- ☐ double distilled (dd) water

Solutions and buffers

Wash Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaOH	0.5 M	39.997	1 M	4 g/ 100 mL	50 mL
NaCl	2.0 M	58.440	5 M	29.2 g/ 100 mL	40 mL
LDAO*	2% (v/v)	229.40	30% (w/v)	0.3 g/ 1 mL	6.6 mL

Instructions: Mix components. Add dd water to a final volume of 100 mL.
Additionally, PureCube NTA, PureCube 100 NTA and PureCube IDA are compatible with the following cleaning reagents: 100% methanol, 100% ethanol, 8 M urea, 6 M guanidinium hydrochloride, 30% (v/v) acetonitrile, 1 M NaOH.

* LDAO is only required if a membrane protein was purified on the resin being washed or regenerated. An alternate detergent may be used but generally we recommend LDAO because it is non-ionic, harsh to proteins, and easily washed off the resin.

100 mM EDTA, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Amount needed
EDTA	100 mM	292.24	2.922 g/ 100 mL

Instructions: Weigh EDTA, fill up to 100 mL dd water and mix well.

10 mM $NiSO_4$, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Amount needed
$NiSO_4$	10 mM	154.75	0.155 g/ 100 mL
Instructions: Weigh $NiSO_4$, fill up to 100 mL dd water and mix well.			

Regeneration Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Acetic acid (97%)	100 mM	60.05	10%=0.17 M	10.3 mL/100 mL	6.05 mL
NaCl	150 mM	58.44	5 M	29.2 g/100 mL	1.5 mL
Instructions: Add components to 80 mL dd water. Mix well and fill up to 100 mL.					

20 mM Tris, pH 8.0, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Amount needed
Tris base	20 mM	121.14	242 mg
Instructions: Dissolve in 80 mL dd water. Set pH to 8.0 using HCl and fill up to 100 mL.			

Procedure

Wash (recommended after each run)

1. Remove the majority of the fluid in the column containing the Ni-NTA or Ni-IDA matrix. Add 10 bv dd water and allow the majority of the water volume to drip out of the column.
2. Add 10 bv Wash Buffer to the column and allow the volume to completely flow through the matrix.
3. Rinse the column again with 10 bv dd water.
4. Add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The matrix is now ready to be re-used.

Note: You can allow the fluid to drip through the column by gravity, or use a pressure bulb to gently force the fluid through the matrix. Ensure not to dry out the matrix.

Wash and regenerate (recommended after each run, latest after 5 runs)

1. Remove the majority of the fluid in the column containing the Ni-NTA or Ni-IDA matrix. Add 10 bv dd water and allow the majority of the volume to drip out of the column.
2. Add 10 bv 100 mM EDTA to the column and allow the entire volume to flow through the matrix.
3. Rinse the column again with 10 bv dd water.
4. Add 10 bv Wash Buffer to the column and allow the entire volume to flow through the matrix.

Note: For removal of contaminations with very hydrophobic proteins or lipids, or precipitated proteins, incubate the matrix with one of the following chemicals for 1-2 h: 100% methanol, 100% ethanol, 8 M urea, 6 M guanidinium hydrochloride, 30% acetonitrile, or 1 M NaOH. Thoroughly wash with distilled water.

5. Rinse the column with 10 bv dd water.
6. Add 10 bv 10m M $NiSO_4$ to recharge the matrix. Allow the volume to drip through the column by gravity.
7. Rinse the column with 5 bv dd water.
8. Add 5 bv of Regeneration Buffer and incubate the column for 15 min at room temperature.
9. Wash twice with 5 bv dd water each.
10. Wash with 5 bv 20 mM Tris pH 8.0.
11. Wash twice with 5 bv dd water each.
12. Add 10 bv of 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The matrix is now ready to be re-used.

Wash and regenerate reduced resins (e.g. after use with DTT)

1. Remove the majority of the fluid in the column containing the Ni-NTA or Ni-IDA resin. Add 10 bv dd water and allow the majority of the volume to drip out of the column.
2. Briefly wash the resin with 10 bv 1–3% (v/v) HCl. Minimize the exposure time of the resin to HCl.
3. Rinse the column with 10 bv dd water.
4. If the resin is not completely white, repeat steps 2 and 3. Otherwise, continue to step 4.
5. Add 10 bv Wash Buffer and allow the majority of the volume to drip out of the column.
6. Rinse the column with 10 bv dd water.
7. Add 10 bv 10 mM $NiSO_4$ to recharge the resin. Allow the volume to drip through the column by gravity.
8. Rinse the column with 5 bv dd water.
9. Add 5 bv of Regeneration Buffer and incubate the matrix for 15 min at room temperature.
10. Wash twice with 5 bv dd water each.
11. Wash with 5 bv 20 mM Tris pH 8.0.
12. Wash twice with 5 bv dd water each.
13. Add 10 bv 20% (v/v) ethanol and allow the majority of the volume to drip out of the column. The matrix is now ready to be re-used.

Note: Resins exposed to reducing agents should always be regenerated after a run.

Note: The concentration of HCl depends on the extent to which the resin is reduced. For example, 1% HCl was sufficient to strip Ni-NTA and Ni-IDA resin exposed to 1 mM DTT, 2% HCl for 5 mM DTT, and 3% for 10 mM DTT.

Note: The extensive wash steps remove free nickel ions from the column, enhancing performance of the material in subsequent purifications, especially in presence of DTT.