Quick-Start Protocol April 2016

Ni-NTA Agarose Purification of 6xHis-tagged Proteins from *E. coli* under Native Conditions

Imidazole stock solution in QIA*express*® Kits (cat. nos. 32149 and 32169) can be stored at 2–8°C for up to 3 months if not otherwise stated on label. Ni-NTA matrices and other kit components can be stored under these conditions for up to 12 months if not otherwise stated on label.

Further information

- *The QIA*expressionist: **www.qiagen.com/HB-2083**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Preparation of cleared *E. coli* lysates under native conditions

Notes before starting

- Prepare a bacterial cell pellet (frozen at –20°C or –80°C).
- Buffer compositions are provided in the appendix of *The QIA*expressionist.
- Lysis may include Benzonase® nuclease (e.g., Novagen cat. no. 70664-3).

1. Thaw cell pellet for 15 min on ice. Resuspend cells in lysis buffer (minimum volume 4 ml) with 10 mM imidazole at 2–5 ml per gram wet weight.

   Note: If the tagged protein does not bind under these conditions, imidazole should be reduced to 1–5 mM. For proteins exhibiting high binding affinities, the imidazole concentration can be increased to 20 mM.
2. Add lysozyme to 1 mg/ml and Benzonase nuclease (3 Units per ml of original cell culture volume processed) and incubate on ice for 30 min.
   For alternative lysis methods, see *The QIA*expressionist.
3. Centrifuge lysate at 10,000 x *g* for 20–30 min at 4°C to pellet the cellular debris. Save supernatant and store on ice.
   Any insoluble material must be solubilized using denaturing conditions before purification under denaturing conditions (see *The QIA*expressionist).

4. Add 5 µl 2x SDS-PAGE sample buffer to 5 µl cleared lysate supernatant. Store at –20°C for SDS-PAGE analysis.

Purification of 6xHis-tagged proteins under native conditions

Notes before starting

- During centrifugation in step 3 of "Preparation of cleared *E. coli* lysates under native conditions" (above), equilibrate the Ni-NTA matrix (step 1).
- Buffer compositions are provided in the appendix of *The QIA*expressionist.

1. Pipet 1 ml of Ni-NTA slurry (0.5 ml bed volume) to a 15 ml tube and briefly centrifuge. Remove supernatant and add 2 ml of lysis buffer. Mix by gently inverting. Repeat the centrifugation step and remove the supernatant.
2. Add 4 ml cleared lysate (from step 3 above) to this equilibrated matrix and mix gently by shaking (200 rpm on a rotary shaker) at 4°C for 60 min.
3. Load the lysate–Ni-NTA mixture into a column with the bottom outlet capped.
4. Remove bottom cap and collect the column flow-through. Save flow-through for SDS-PAGE analysis.
5. Wash twice with 5 bed volume (2.5 ml) wash buffer. Collect wash fractions for SDS-PAGE analysis.
6. Elute the protein 4 times with 0.5 ml elution buffer. Collect the eluate in four tubes and analyze by SDS-PAGE.

General suggestions

- The composition of the lysis, wash, and elution buffers can be modified to suit the particular application (see *The QIA*expressionist).
- Keep cells and protein solutions at 0–4°C at all times to prevent protein degradation. The addition of protease inhibitors may also be necessary.
- Low concentrations of imidazole in lysis and wash buffers minimize nonspecific binding and reduce the amount of contaminating proteins.

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