Quick-Start Protocol March 2016

Automated Purification of 6xHis-tagged Proteins from *E. coli* Using Ni-NTA Superflow 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

- *Ni-NTA Superflow Cartridge Handbook*: **www.qiagen.com/HB-0885**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare bacterial cell pellet (40–200 ml culture; 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).

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

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, 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.

Notes before starting

- Buffer compositions are provided in the appendix of *The QIA*expressionist.

Automated purification of 6xHis-tagged proteins under native conditions

1. Assemble the column according to the manufacturer's instructions. Remove the top adapter of the column and cap the bottom outlet. Alternatively, use QIAGEN Ni-NTA Superflow Cartridges (cat. nos. 30760 and 30721) and continue with step 5.
2. Completely resuspend the 50% Ni-NTA Superflow slurry and pour the slurry into the column.

   Note: Avoid introducing air bubbles. Slowly pour the slurry down a thin glass rod inserted into the empty column.
3. Allow the resin to settle. Do not allow the resin to dry.
4. Insert top adapter and adjust to top of bed. Do not trap any air bubbles. The column can now be connected to the system.
5. Equilibrate column with 5 column volumes of lysis buffer.

   Note: The recommended flow rate is 170 cm/h (1 ml/min for the 1 ml cartridge or 5 ml/min for the 5 ml cartridge). Monitor elution at 280 nm; the baseline should be stable after washing with 5 column volumes.
6. Apply lysate to column and wash with lysis buffer until the A_{280} is stable. Usually, 5–10 column volumes are sufficient.
7. Wash with wash buffer until the A_{280} is stable. Usually, 10 column volumes are sufficient. Collect fractions for SDS-PAGE analysis.
8. Elute the protein with elution buffer.

   Note: Imidazole absorbs at 280 nm. If small amounts of 6xHis-tagged proteins are purified, elution peaks may be poorly visible.

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