Quick-Start Protocol March 2016

Ni-NTA Spin Column Purification of 6xHis-Tagged Proteins under Native Conditions from *E. coli* Cell Lysates

The Ni-NTA Spin Kit (cat. no. 31314) and Ni-NTA Spin Columns (cat. no. 31014) can be stored at 2–8°C for up to 18 months if not otherwise stated on label.

Further information

- *Ni-NTA Spin Kit Handbook*: **www.qiagen.com/HB-0883**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare bacterial cell pellet (stored at –20°C or –80°C).
- Buffer compositions are provided in the appendix of the *Ni-NTA Spin Kit Handbook*.
- Lysis requires Benzonase® nuclease (e.g., Novagen cat. no. 70664-3).

1. Resuspend a pellet derived from 5 ml cell culture volume in 630 µl Lysis Buffer (NPI-10). Add 70 µl Lysozyme Stock Solution (10 mg/ml) and add 3 Units/ml culture volume Benzonase Nuclease (i.e., for cell pellets from 5 ml cultures, add 15 Units Benzonase Nuclease).
2. Incubate on ice for 15–30 min.
3. Centrifuge lysate at 12,000 x *g* for 15–30 min at 4°C. Collect supernatant.

   Note: Save 20 µl of the cleared lysate for SDS-PAGE analysis.

4. Equilibrate the Ni-NTA spin column with 600 µl Buffer NPI-10. Centrifuge for 2 min at 890 x *g* (approx. 2900 rpm).

   Note: The spin columns should be centrifuged with an open lid to ensure that the centrifugation step is completed under 2 min.

5. Load up to 600 µl of the cleared lysate containing the 6xHis-tagged protein onto the pre-equilibrated Ni-NTA spin column. Centrifuge for 5 min at 270 x *g* (approx. 1600 rpm), and collect the flow-through.

   Note: To ensure efficient binding, do not exceed 270 x *g* when centrifuging Ni-NTA spin columns. The spin columns can be centrifuged with an open lid.

   Note: Save the flow-through for analysis by SDS-PAGE.

6. Wash the Ni-NTA spin column twice with 600 µl Buffer NPI-20. Centrifuge for 2 min at 890 x *g* (approx. 2900 rpm).

   Note: Save the flow-through for analysis by SDS-PAGE.

7. Elute the protein twice with 300 µl Buffer NPI-500. Centrifuge for 2 min at 890 x *g* (approx. 2900 rpm), and collect the eluate.

   Note: Most of the 6xHis-tagged protein (>80%) should elute in the first 300 µl eluate. The remainder will elute in the second 300 µl.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.