Quick-Start Protocol April 2016

Purification of 6xHis-tagged Proteins in Single Reaction Tubes with Ni-NTA Magnetic Agarose Beads

Ni-NTA Magnetic Agarose Beads (cat. nos. 36111 and 36113) are supplied in a 5% (v/v) suspension in 30% ethanol and should be stored at 2–8°C. They can be stored under these conditions for up to 6 months if not otherwise stated on label.

Further information

- Ni-NTA Magnetic Agarose Beads Handbook: **www.qiagen.com/HB-2095**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

Cell lysis under native conditions

- Prepare bacterial pellets (frozen at –20 or –80°C).
- Lysis includes Benzonase® nuclease (e.g., Novagen cat. no. 70664-3).
- For buffer compositions, see appendix in *Ni-NTA Magnetic Agarose Beads Handbook*.

1. Thaw cells for 15 min and resuspend in Lysis Buffer-Tween (0.5 ml per 5 ml original culture volume).
2. Prepare crude or cleared lysates by following one of these procedures.
   - 2a. Add lysozyme to 1 mg/ml and Benzonase nuclease at 3 Units per ml culture volume. Incubate on ice. Use the crude lysate without further clarification.
   - 2b. Add lysozyme plus RNase A (10 µg/ml) and DNase I (5 µg/ml) and incubate on ice for 30 min. Sonicate on ice using a sonicator equipped with a microtip. Clear lysate by centrifugation at 10,000 x *g* for 30 min at 4°C. Collect supernatant (i.e., the cleared lysate).

2c. Add lysozyme and incubate on ice for 30 min. Draw the lysate through a narrow-gauge blunt-ended syringe needle several times. Clear lysate by centrifugation at 10,000 x *g* for 30 min at 4°C. Collect supernatant.

Note: When using 96-well blocks, incubation can be at room temperature for 15 min; 96-well blocks can be centrifuged at 5000 x *g* for 30 min.

Purification in single reaction tubes

Notes before starting

- For buffer compositions, see appendix in *Ni-NTA Magnetic Agarose Beads Handbook*.

1. Resuspend the Ni-NTA Magnetic Agarose Beads by vortexing for 2 s and then immediately add 200 µl of the 5% Ni-NTA Magnetic Agarose Bead suspension to 1 ml of the lysate containing the 6xHis-tagged protein.
2. Mix the suspension gently on an end-over-end shaker for 30 min to 1 h at room temperature (15–25°C).

   It may be necessary to incubate at 4°C if the protein is not stable at room temperature.
3. Place the tube on a magnetic separator for 1 min and remove supernatant with a pipet.
4. Remove tube from the magnet, add 500 µl of wash buffer, mix the suspension, place the tube on a magnetic separator for 1 min and remove wash buffer.
5. Repeat step 4 another 1–2 times.

   Buffer remaining after the final wash should be removed completely.
6. Add 100 µl of elution buffer, mix the suspension, incubate the tube for 1 min, place for 1 min on magnetic separator and collect the eluate.
7. Repeat step 6.

Scan QR code for handbook.

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