QIAGEN Supplementary Protocol:

Manual purification of 6xHis-tagged proteins from *E. coli* using Ni-NTA Superflow Columns

The protocols given below are for use with Ni-NTA Superflow Columns (1.5 ml) in manual procedures. Ni-NTA Superflow Columns have been specially designed and optimized for both manual applications and for automated large-scale purification of 6xHis-tagged proteins on QIAGEN® BioRobot® systems. For more details of the advantages of BioRobot systems see the *Ni-NTA Superflow BioRobot Handbook* supplied with the columns or contact one of the QIAGEN Technical Service Departments or local distributors listed on the last page of the handbook.

The following protocols have been designed for the use of Ni-NTA Superflow Columns on the QIAvac 6S vacuum manifold or in gravity-flow applications on the QIArack. Up to 15 mg 6xHis-tagged protein can be purified per column from cleared lysate derived from up to 1 liter of (*E. coli*) bacterial culture.

For the vacuum method, we strongly recommend using a vacuum source that can be conveniently regulated to provide vacuum pressures of between –10 and –100 mbar. The vacuum setup should include a waste trap between the vacuum manifold and the pump.

Please read the *Ni-NTA Superflow BioRobot Handbook* carefully before beginning the vacuum-driven procedure. *The* QIA*expressionist*™ is a comprehensive handbook for Ni-NTA affinity purification in general. Both handbooks are supplied with the Ni-NTA Superflow Columns (1.5 ml). All buffers used in these protocols are described in the handbooks.

IMPORTANT: Please consult the Safety Information section in the *Ni-NTA Superflow BioRobot Handbook* before beginning this procedure.

Protocol 1. Purification of 6xHis-tagged proteins from *E. coli* under native conditions

The following cell lysis and protein purification procedures should be performed at a constant temperature (either at 4°C or at room temperature, 15–25°C), to avoid repeated temperature shifts that may be harmful to protein activity and structure.

Reagents and equipment to be supplied by user

- Buffer NPI-10
- Lysozyme
- Benzonase®

Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com.

Cell lysis and generation of cleared lysates

1. **Grow cell cultures, induce protein expression, culture for a previously optimized time period, harvest the cells by centrifugation, and store the pellets at –20°C or at –70°C for at least 1 h.**

   For details, see the Cultivation of *E. coli* M15[pREP4] Harboring pQE Expression Constructs protocol in the *Ni-NTA Superflow BioRobot Handbook*.

2. **Place frozen bacterial cells at room temperature and allow to thaw for 15 min.**
3. **Add 10 ml buffer NPI-10 and 1 ml lysozyme solution (10 mg/ml) to the thawed cells. In addition, add 3 units Benzonase for every ml of the original cell culture volume (for example, for a 100 ml cell culture, add 300 units Benzonase).**

   Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com .
4. **Resuspend the pellet by pipetting up and down.**
5. **Incubate for 30 min at either 4°C or room temperature (15–25°C).**
6. **Transfer crude lysate into appropriate tubes and centrifuge for 30 min at 15,000 x *g* at 4°C. Insoluble cell components will be pelleted at the bottom of the tube.**
7. **Collect supernatants containing soluble 6xHis-tagged proteins and transfer into a fresh tube.**
8. **Continue with the protein purification by following either procedure A (using the QIAvac 6S) or procedure B (using gravity flow), below.**

A: Protein purification under native conditions using the QIAvac 6S

Reagents and equipment to be supplied by user

- QIAvac 6S (cat. no. 19503) and QIAvac Luer Adapter Set (cat. no. 19541)
- 24-well elution vessel (e.g., 24-Well Blocks RB, cat. no. 19583)
- Buffers NPI-10, NPI-20, and NPI-250

Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com.

1. **During cell lysis, prepare the QIAvac 6S vacuum manifold.**
2. **Position the required number of Ni-NTA Superflow Columns on the Luer Adapter strips on the QIAvac 6S top plate. Note: first break the seals at the outlet of the columns before opening the screw cap! Ensure that columns fit tightly by twisting when inserting them into the Luer Adapters.**

   For positioning, refer to the figure "Positioning of Columns on the Vacuum Manifold" (Figure 6, page 47) in the *Ni-NTA Superflow BioRobot Handbook* (September 2002 edition).

   Before use, Ni-NTA Superflow Columns should have been stored in an upright position. Check that the resin is contained in the narrow part of the column body before opening the columns. If the resin is attached to the sides or to the cap of the column, resuspend the resin by inverting the column and allow resin to settle before proceeding with step 3.
3. **Remove the storage buffer from above the resin either by using a pipet, gravity flow, or by applying a weak vacuum (approximately –10 mbar for 4 min or until buffer has been drawn through).**

   Avoid allowing the columns to run dry.*

* *If movement of liquid through the columns appears unsynchronized, stop the vacuum when buffer has been drawn through the first column and allow the liquid in the remaining columns to drain by gravity flow. The columns will not run dry if left to drain by gravity flow, but they will run dry following continued application of a vacuum. The Ni-NTA resin turns white when the column runs dry and when air is drawn through the resin. If a column runs dry, continue with the procedure, as protein purification will not be significantly affected. However, activity of extremely oxidation-sensitive proteins may be decreased if excess air has been drawn through the column.*

4. **Equilibrate the columns by adding 10 ml Buffer NPI-10 to each column, and apply a vacuum of approximately –10 mbar for 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

5. **Transfer the cleared lysates into the equilibrated columns, and apply a vacuum of approximately –10 mbar for 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

6. **Perform the first wash step by pipetting 10 ml Buffer NPI-20 into each column, and apply a vacuum of approximately –10 mbar for 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

7. **Perform a second wash step by repeating step 6.**

   Very rarely, imidazole concentrations of 20 mM can interfere with binding of 6xHis-tagged proteins to the resin. If binding is inefficient, reduce the imidazole concentration in the wash buffer (e.g., to 10 mM).

8. **Place the 24-well elution vessel inside the QIAvac 6S base.**

9. **To elute the 6xHis-tagged proteins, pipet 3 ml Buffer NPI-250 into each column, and apply a vacuum of approximately –10 mbar for approximately 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

   Approximately 80% of the bound 6xHis-tagged protein is eluted within the first fraction. If desired, a second elution step can be performed to increase recovery, by repeating step 9. The second eluate can be collected into the same 24-well vessel or into a second 24-well elution vessel.

B: Protein purification under native conditions using gravity flow

Reagents and equipment to be supplied by user

- QIArack (cat. no. 19015)
- Elution vessels (e.g., 4–14 ml polypropylene tubes)
- Buffers NPI-10, NPI-20, and NPI-250

Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com.

1. **Position the required number of Ni-NTA Superflow Columns (1.5 ml) on the QIArack. Note: first break the seals at the outlet of the columns before opening the screw cap!**

   Before use, Ni-NTA Superflow Columns should have been stored in an upright position. Check that the resin is contained in the narrow part of the column body before opening the columns. If the resin is attached to the sides or to the cap of the column, resuspend the resin by inverting the column and allow resin to settle before proceeding with step 2.

2. **Remove the storage buffer from above the resin either by using a pipet or by allowing it to drain through by gravity flow.**

   The columns will not run dry by gravity flow.

3. **Equilibrate the columns by pipetting 10 ml Buffer NPI-10 into each column, and allow buffer to drain through completely by gravity flow.**

4. **Transfer the cleared lysates into the equilibrated columns and allow the columns to drain by gravity flow.**
5. **Perform the first wash step by pipetting 10 ml Buffer NPI-20 into each column. Allow the buffer to drain through completely by gravity flow.**
6. **Perform a second wash step by repeating step 5.**

   Very rarely, imidazole concentrations of 20 mM can interfere with binding of 6xHis-tagged proteins to the resin. If binding is inefficient, reduce the imidazole concentration in the wash buffer (e.g., to 10 mM).
7. **Place an elution vessel under each column outlet.**
8. **To elute the 6xHis-tagged proteins, add 3 ml Buffer NPI-250 to each column, allow buffer to flow through completely, and collect flow-through in the elution vessels.**

   Approximately 80% of the bound 6xHis-tagged protein is eluted within the first fraction. If desired, a second elution step can be performed to increase recovery, by repeating step 8. The second eluate can be collected into the same or into a second elution vessel.

Protocol 2: Purification of 6xHis-tagged proteins from *E. coli* under denaturing conditions

Under denaturing conditions, the entire lysis and purification procedures should be performed at room temperature (15–25°C).

Reagents and equipment to be supplied by user

- Buffer B-7 M urea

Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com.

Cell lysis and generation of cleared lysates

1. **Grow cell cultures, induce protein expression, culture for a previously optimized time period, harvest the cells by centrifugation, and store the pellets at –20°C or at –70°C for at least 1 h.**

   For details, see the Cultivation of *E. coli* M15[pREP4] Harboring pQE Expression Constructs protocol in the *Ni-NTA Superflow BioRobot Handbook*.
2. **Place frozen bacterial cells at room temperature and allow to thaw for 15 min.**
3. **Add 10 ml Buffer B–7 M urea to the thawed cells. In addition, add 3 units Benzonase for every ml of the original cell culture volume (for example, for a 100 ml cell culture, add 300 units Benzonase).**
4. **Resuspend the pellet by pipetting up and down.**
5. **Incubate for 30 min at room temperature.**
6. **Transfer crude lysate into appropriate tubes and centrifuge for 15 min at 15,000 x *g* at room temperature (15–25°C). Insoluble cell components will be pelleted at the bottom of the tube.**
7. **Collect supernatant containing solubilized 6xHis-tagged proteins and transfer into a fresh tube.**
8. **Continue with the protein purification by following either procedure A (using the QIAvac 6S) or procedure B (using gravity flow), below.**

A: Protein purification under denaturing conditions using the QIAvac 6S

Reagents and equipment to be supplied by user

- QIAvac 6S (cat. no. 19503)
- QIAvac Luer Adapter Set (cat. no. 19541)
- 24-well elution vessel (e.g., 24-Well Blocks RB, cat. no. 19583)
- Buffer B-7 M urea, Buffer C-7 M urea, Buffer E-8 M urea

Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com.

1. **During cell lysis, prepare the QIAvac 6S vacuum manifold.**
2. **Position the required number of Ni-NTA Superflow Columns on the Luer Adapter strips on the QIAvac 6S top plate. Note: first break the seals at the outlet of the columns before opening the screw cap! Ensure that columns fit tightly by twisting when inserting them into the Luer Adapters.**

   For positioning, refer to the figure "Positioning of Columns on the Vacuum Manifold" (Figure 6, page 47) in the *Ni-NTA Superflow BioRobot Handbook* (September 2002 edition).

   Before use, Ni-NTA Superflow Columns should have been stored in an upright position. Check that the resin is contained in the narrow part of the column body before opening the columns. If the resin is attached to the sides or to the cap of the column, resuspend the resin by inverting the column and allow resin to settle before proceeding with step 3.
3. **Remove the storage buffer from above the resin either by using a pipet, gravity flow, or by applying a weak vacuum (approximately –10 mbar for 4 min or until buffer has been drawn through)**

   Avoid allowing the columns to run dry.*
4. **Equilibrate the columns by pipetting 10 ml of Buffer B–7 M urea into each column, and apply a vacuum of approximately –10 mbar for approximately 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.
5. **Transfer the cleared lysates into the equilibrated columns, and apply a vacuum of approximately –10 mbar for approximately 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.
6. **Perform the first wash step by pipetting 10 ml Buffer B–7 M urea into each column, and apply a vacuum of approximately –10 mbar for approximately 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

* *If movement of liquid through the columns appears unsynchronized, stop the vacuum when buffer has been drawn through the first column and allow the liquid in the remaining columns to drain by gravity flow. The columns will not run dry if left to drain by gravity flow, but they will run dry following continued application of a vacuum. The Ni-NTA resin turns white when the column runs dry and when air is drawn through the resin. If a column runs dry, continue with the procedure, as protein purification will not be significantly affected. However, activity of extremely oxidation-sensitive proteins may be decreased if excess air has been drawn through the column.*

7. **Perform a second wash step by pipetting 10 ml Buffer C–7 M urea into each column, and apply a vacuum of approximately –10 mbar for approximately 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

8. **Place the 24-well elution vessel inside the QIAvac 6S base.**

9. **To elute the 6xHis-tagged proteins, pipet 3 ml Buffer E–8 M urea into each column, and apply a vacuum of approximately –10 mbar for approximately 4 min or until buffer has been drawn through.**

   Avoid allowing the columns to run dry.

   Approximately 80% of the bound 6xHis-tagged protein is eluted within the first fraction. If desired, a second elution step can be performed to increase recovery, by repeating step 9. The second eluate can be collected into the same 24-well vessel or into a second 24-well elution vessel.

B: Protein purification under denaturing conditions using gravity flow

Reagents and equipment to be supplied by user

- QIArack (cat. no. 19015)
- Elution vessels (e.g., 4–14 ml polypropylene tubes)
- Buffer B-7 M urea, Buffer C-7 M urea, Buffer E-8 M urea

Buffer compositions can be found in the *Ni-NTA Superflow BioRobot Handbook*, which can be downloaded in convenient PDF format at www.qiagen.com.

1. **Position the required number of Ni-NTA Superflow Columns on the QIArack. Note: first break the seals at the outlet of the columns before opening the screw cap!**

   Before use, Ni-NTA Superflow Columns should have been stored in an upright position. Check that the resin is contained in the narrow part of the column body before opening the columns. If the resin is attached to the sides or to the cap of the column, resuspend the resin by inverting the column and allow resin to settle before proceeding with step 2.

2. **Remove the storage buffer from above the resin either by using a pipet or by allowing it to drain through by gravity flow.**

   The columns will not run dry by gravity flow.

3. **Equilibrate the columns by pipetting 10 ml Buffer B–7 M urea into each column, and allow buffer to drain through completely by gravity flow.**
4. **Transfer the cleared lysates into the equilibrated columns and allow the columns to drain by gravity flow.**
5. **Perform the first wash step by pipetting 10 ml Buffer B–7 M urea into each column. Allow the buffer to drain through completely by gravity flow.**
6. **Perform a second wash step by pipetting 10 ml Buffer C–7 M urea into each column. Allow the buffer to drain through completely by gravity flow.**
7. **Place an elution vessel under each column outlet.**
8. **To elute the 6xHis-tagged proteins, pipet 3 ml Buffer E–8 M urea into each column, allow buffer to flow through completely, and collect flow-through in the elution vessels.**

   Approximately 80% of the bound 6xHis-tagged protein is eluted within the first fraction. If desired, a second elution step can be performed to increase recovery, by repeating step 8. The second eluate can be collected into the same or into a second elution vessel.