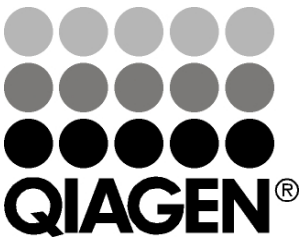
July 2011

Ni-NTA Superflow Cartridge Handbook

For manual or FPLC™ purification of
His-tagged proteins



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

Cat. no.	Ni-NTA Superflow Cartridges (1 ml)	Ni-NTA Superflow Cartridges (5 ml)	Handbook
30721	5		1
30725	100		1
30760		1	1
30761		5	1
30765		100	1

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding Ni-NTA Superflow Cartridges or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside back cover).

Storage and Stability

Ni-NTA Superflow Cartridges should be stored at 2–8°C. Do not freeze! Cartridges can be stored under these conditions for one year without any reduction in performance.

Product Use Limitations

The Ni-NTA Superflow Cartridge is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see inside back cover).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

The following risk and safety phrases apply to **Ni-NTA Superflow Cartridges**: Contains ethanol and nickel-nitrilotriacetic acid. Harmful, sensitizer, and flammable. Risk and safety phrases:* R10-22-40-42/43. S13-26-36-46.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from: Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable. R22: Harmful if swallowed. R40: Limited evidence of a carcinogenic effect. R42/43: May cause sensitization by inhalation and skin contact. S13: Keep away from food, drink and animal feedingstuffs. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36: Wear suitable protective clothing. S46: If swallowed, seek medical advice immediately and show the container or label.

Introduction

QIAGEN Ni-NTA Superflow Cartridges are pre-filled with 1 ml or 5 ml Ni-NTA Superflow and are ready to use for purification of 6xHis-tagged proteins using a syringe, peristaltic pump, or liquid chromatography system (such as the ÄKTAdesign™ or FPLC™ System).

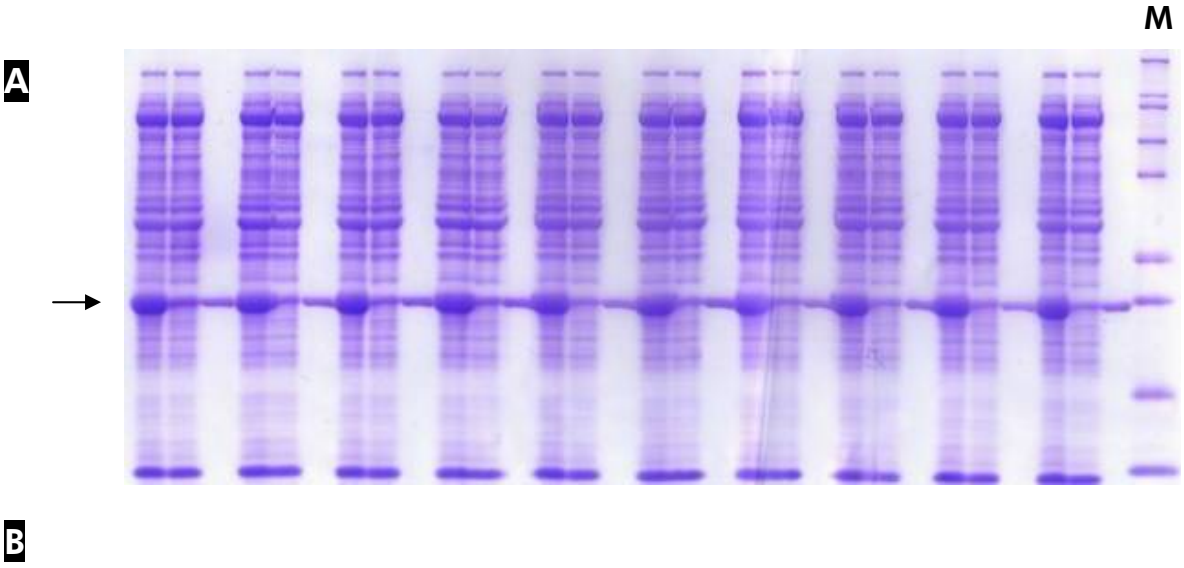


Figure 1. Highly reproducible and reliable purification. **A** His-tagged pGAPase (arrowed) was purified in 10 sequential purification procedures. Column load was 10 ml aliquots of cleared *E. coli* cell lysate containing 30 mg spiked protein. Between purification runs the column was cleaned in place using 0.5 M NaOH. Groups of samples show column load, flow-through, and peak elution fraction. **M**: markers. **B** Overlaid chromatogram traces of the 10 purification runs .

Ni-NTA Superflow Cartridge Specifications

	1 ml Cartridge	5 ml Cartridge
Support	Superflow (highly cross-linked 6% agarose)	
Bead diameter	60–169 µm	
Column dimensions (mm i.d.)	6.7 mm x 28.0 mm	14.7 mm x 29.8 mm
Maximum pressure*	5 bar, 0.5 MPa	5 bar, 0.5 MPa
Typical back pressure (Buffer NPI-10, 10% glycerol)	1.0 bar, 0.1 MPa (1 ml/min)	2.0 bar, 0.2 MPa (5 ml/min)
Recommended flow rate	1 ml/min (155 cm/h)	5 ml/min (170 cm/h)
Maximum flow rate†	10 ml/min (1560 cm/h)	40 ml/min (1360 cm/h)
Column connections	See Table 3, page 14	See Table 3, page 14
pH stability short term (≤ 2h)	2–14	2–14
pH stability long term (> 2h)	3–12	3–12
Binding capacity‡	At least 50 mg (up to 1 µmol @ 20 kDa)	At least 250 mg (up to 5 µmol @ 20 kDa)
System compatibility	Automated chromatography systems (e.g., ÄKTA, FPLC, BioLogic, BioCAD, Vision workstation)	
Cartridge body material	Polypropylene	
Connectors	1/16" (inlet); M6 (outlet)	

* The maximum pressure usable with the Superflow matrix itself is 10 bar. However, stability of the Cartridges is only guaranteed up to 5 bar.

† High flow rates may lead to reduced recovery of 6xHis-tagged protein.

‡ Determined for a monomeric 30 kDa globular 6xHis-tagged protein. Binding capacity may vary from protein to protein.

The QIA*express*® System

The QIA*express* System is based on the 6xHis tag, an affinity tag comprising six consecutive histidine residues. This affinity tag binds with remarkable selectivity and affinity to QIAGEN's exclusive, patented nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography matrices. The unique features of the QIA*express* System provide a number of significant advantages (Table 1) that are not available with other affinity-tag and chromatography methods.

Table 1. Features and benefits of the QIA*express* System

Feature	Benefits
High affinity and selectivity of binding between the 6xHis tag and Ni-NTA	High purity protein in a single-step standardized purification procedure.
The interaction of the 6xHis tag with Ni-NTA matrices is conformation independent	One-step purification can be carried out under native or denaturing conditions.
Mild elution conditions can be used	Binding, washing, and elution are highly reproducible, and have no effect on protein structure. Pure protein products are ready for direct use in downstream applications.
The 6xHis tag is much smaller than other commonly used tags	Tag does not interfere with the structure and function of the recombinant protein.
The 6xHis tag is uncharged at physiological pH	The 6xHis tag does not interfere with secretion.
The 6xHis tag is poorly immunogenic	The recombinant protein can be used without prior removal of the tag as an antigen to generate antibodies against the protein of interest.

Ni-NTA Superflow

Ni-NTA Superflow is comprised of Ni-NTA coupled to Superflow resin. It combines superior mechanical stability with outstanding flow characteristics and high dynamic binding capacity. The capacity for 6xHis-tagged proteins is up to 50 mg/ml. This resin allows one-step purification of 6xHis-tagged proteins using high flow rates and pressures for efficient production-scale and FPLC applications. Ni-NTA is remarkably robust and is compatible with a wide range of reagents, such as 2 M NaCl, 10 mM DTT, 8 M urea, and many detergents (see Table 2).

Limitations

Ni-NTA matrices should not be exposed to high concentrations of strong reducing agents such as DTT or DTE; in high concentrations these reagents reduce nickel ions and may prevent them from binding 6xHis-tagged proteins. Ni-NTA resins turn brown in the presence of reducing agents. In most situations, β-mercaptoethanol can be used at concentrations up to 20 mM and DTT compatibility has been demonstrated up to a concentration of 10 mM.

EDTA, EGTA, or any other strong chelating agents bind nickel ions and strip them from the NTA matrices. NTA resins become white in the absence of nickel ions. Use any reducing or chelating agent with care, and if in doubt, test it on a small amount of Ni-NTA resin. High concentrations of buffer components containing strong electron-donating groups or amino acids such as arginine, glutamine, glycine, or histidine in the lysate should also be avoided.

Cells should be lysed without the use of strong chelating agents such as EDTA, strong reducing agents such as DTT, or ionic detergents such as SDS. Although there are instances in which small amounts of these reagents have been used successfully, we do not recommend their use.

For more detailed information, see Table 2.

Table 2. Compatibility of reagents with Ni-NTA Superflow

Reagent	Effect	Comments
Buffer reagents		
Tris, HEPES, MOPS	Buffers with secondary or tertiary amines may reduce nickel ions	Up to 100 mM can be used, however sodium phosphate buffer is recommended
Chelating reagents		
EDTA, EGTA	Strip nickel ions from resin	Up to 1 mM has been used successfully in some cases, but care must be taken
Sulfhydril reagents		
β-mercaptoethanol	Prevents disulfide cross-linkages. Can reduce nickel ions at higher concentration	Up to 20 mM can be used. Do not store resin under reducing conditions
DTT, DTE	At high concentrations (>1 mM) resin may turn reversibly brown due to nickel reduction. Up to 10 mM has been tested and shown not to compromise purification or increase nickel leaching.	Up to 10 mM DTT has been used successfully. Do not store resin under reducing conditions.
Detergents		
Nonionic detergents (Triton, Tween, NP-40, etc.)	Removes background proteins and nucleic acids	Up to 2% can be used
Cationic detergents		Up to 1% can be used
CHAPS		Up to 1% can be used
Anionic detergents (SDS, sarkosyl)		Not recommended, but up to 0.3% has been used successfully in some cases
Triton X-114	Removes endotoxins	Up to 2% can be used

Reagent	Effect	Comments
Denaturants		
GuHCl	Solubilize proteins	Up to 6 M
Urea		Up to 8 M
Amino acids		
Glycine		Not recommended
Glutamine		Not recommended
Arginine		Not recommended
Histidine	Binds to Ni-NTA and competes with histidine residues in the 6xHis tag	Can be used at low concentrations (20 mM) to inhibit non specific binding and, at higher concentrations (>100 mM), to elute the 6xHis-tagged protein from the Ni-NTA matrix
Other additives		
NaCl	Prevents ionic interactions	Up to 2 M can be used at least 300 mM should be used
$MgCl_2$		Up to 4 M
$CaCl_2$		Up to 5 mM
Glycerol	Prevents hydrophobic interaction between proteins	Up to 50%
Ethanol	Prevents hydrophobic interactions between proteins	Up to 20%
BugBuster® Protein Extraction Reagent		Use as recommended

Reagent	Effect	Comments
Imidazole	Binds to Ni-NTA and competes with histidine residues in the 6xHis tag	Can be used at low concentrations (20 mM) to inhibit non specific binding and, at higher concentrations (>100 mM), to elute the 6xHis-tagged protein from the Ni-NTA matrix
Sodium bicarbonate		Not recommended
Hemoglobin		Not recommended
Ammonium		Not recommended
Citrate		Up to 60 mM has been used successfully

Cartridge Connections

Ni-NTA Superflow Cartridges can be used for purification of proteins in a manual procedure (using a syringe) or an automated procedure (using a chromatography system (such as the ÄKTAdesign™ or FPLC™ System). The cartridge inlet and outlet dimensions and required connectors and adapters for manual and automated procedures are detailed in the table below.

Table 3. Connectors Required for Ni-NTA Superflow Cartridges

	Inlet	**Outlet**
Ni-NTA Cartridge	1/16″ female (ÄKTAdesign)	M6 male (FPLC)
Adapters for manual procedure using a syringe	1/16″ male/luer female (e.g., Amersham Product Code 18-1112-51)	
Connector for automated procedure (ÄKTAdesign 1/16″ connectors)	No adapter required	Union M6 female / 1/16″ female (e.g., Amersham Product Code 18-1123-94)
Connector for automated procedure (M6 fittings, [FPLC])	Union M6 female / 1/16″ male (e.g., Amersham Product Code 18-3858-01)	SRTC-2, M6 female (0.5 mm i.d.) (e.g., Amersham Product Code 18-3856-01)

Purification Under Native or Denaturing Conditions

The decision whether to purify 6xHis-tagged proteins under native or denaturing conditions depends on protein location and solubility, the accessibility of the 6xHis tag, the downstream application, and whether biological activity must be retained. Furthermore, if efficient renaturing procedures are available, denaturing purification and subsequent refolding may be considered.

Purification under native conditions

If purification under native conditions is preferred or necessary, the 6xHis-tagged protein must be soluble. However, even when most of the protein is present in inclusion bodies, there is generally some soluble material that can be purified in its native form. The potential for unrelated, non-tagged proteins to interact with the Ni-NTA resin is usually higher under native than under denaturing conditions. This is reflected in the larger number of proteins that appear in the first wash. Nonspecific binding is reduced by including a low concentration of imidazole in the lysis and wash buffers.

In rare cases the 6xHis tag is hidden by the tertiary structure of the native protein, so that soluble proteins require denaturation before they can be purified on Ni-NTA. As a control, a parallel purification under denaturing conditions should always be carried out: If purification is only possible under denaturing conditions, the tag can generally be made accessible by moving it to the opposite terminus of the protein.

Purification under denaturing conditions

High levels of expression of recombinant proteins in a variety of expression systems can lead to the formation of insoluble aggregates; in *E. coli*, these are known as inclusion bodies. A denaturing buffer containing 8 M urea or 6 M guanidine hydrochloride (Gdn·HCl) usually completely solubilizes inclusion bodies and 6xHis-tagged proteins. Under denaturing conditions, the 6xHis tag on a protein will be fully exposed so that binding to the Ni-NTA matrix will improve and the efficiency of the purification procedure will be maximized by reducing the potential for nonspecific binding.

6xHis-tagged proteins purified under denaturing conditions can be used directly, or may have to be renatured and refolded. Protein renaturation and refolding can be carried out on the Ni-NTA cartridge itself prior to elution, or in solution; suggestions can be found in *The QIA*expressionist®.

Protocol: Preparation of Cleared *E. coli* Lysates Under Native Conditions

Materials and reagents to be supplied by user

- Cell pellet
- Buffer NPI-10
- Lysozyme
- Benzonase® Nuclease (purity grade I, 25 U/ml, Merck, Germany, cat. no. 1.0169.0001)
- 2x SDS-PAGE sample buffer
- Optional: Sonicator

Buffer compositions are provided in Appendix A on page 25.

Procedure

1. **Thaw the cell pellet for 15 min on ice and resuspend the cells in Buffer NPI-10 at 2–5 ml per gram wet weight.**

   The amount of cells required depends on the expression level of the 6xHis-tagged protein and the expression system used. The binding capacity of Ni-NTA resins is protein-dependent and normally lies around 50 mg/ml. Buffer NPI-10 contains 10 mM imidazole to minimize binding of untagged, contaminating proteins and increase purity with fewer wash steps. If the tagged protein does not bind under these conditions, the amount of imidazole should be reduced to 1–5 mM. With 6xHis-tagged proteins exhibiting high binding affinities, the imidazole concentration can be increased to 20 mM.

2. **Add lysozyme to 1 mg/ml (50, 000 units/ml) and Benzonase® Nuclease (3 U per ml of original culture volume processed) and incubate on ice for 30 min.**

   Alternatively, add RNase A (10 µg/ml) and DNase I (5 µg/ml) and incubate on ice for 10–15 min or draw the lysate through a narrow-gauge blunt-ended syringe needle several times.

2a. **(Optional) Sonicate on ice using a sonicator equipped with a microtip.**

   Use six 10 s bursts at 200–300 W with a 10 s cooling period between each burst.

3. **Centrifuge lysate at 10,000 x *g* for 20–30 min at 4°C to pellet the cellular debris and save supernatant.**

   A certain proportion of the cellular protein, including the 6xHis-tagged protein, may remain insoluble and will be located in the pellet. For more complete recovery of the tagged protein, this material must be solubilized using denaturing conditions before purification under denaturing conditions.

4. **Add 5 µl 2x SDS-PAGE sample buffer to 5 µl supernatant and store at –20°C for SDS-PAGE analysis.**
5. **Proceed to purification protocol (page 21 or 22).**

Protocol: Preparation of Cleared *E. coli* Lysates Under Denaturing Conditions

Materials and reagents to be supplied by user

- Cell pellet
- 2x SDS-PAGE sample buffer
- Buffer B
- Optional: Buffer B/7 M urea and Benzonase® Nuclease (purity grade I, 25 U/ml, Merck, Germany, cat. no. 1.0169.0001)

Buffer compositions are provided in Appendix A on page 25.

Procedure

1. **Thaw the cell pellet for 15 min on ice and resuspend in Buffer B at 5 ml per gram wet weight.**

1a. **(Optional) Thaw the cell pellet for 15 min on ice, resuspend in Buffer B/7 M urea at 5 ml per gram wet weight, and Benzonase® Nuclease* (3 U per ml of original culture volume processed) and incubate for 30 min at room temperature (20–25°C).**

   The amount of cells required depends on the expression level of the 6xHis-tagged protein and the expression system used. The binding capacity of Ni-NTA resins is protein-dependent and normally lies around 50 mg/ml.

2. **Stir cells for 15–60 min at room temperature or lyse them by gently vortexing, taking care to avoid foaming.**

   Lysis is complete when the solution becomes translucent.

3. **Centrifuge lysate at 10,000 x *g* for 20–30 min at room temperature to pellet the cellular debris.**

   Save supernatant (cleared lysate).

4. **Add 5 µl 2x SDS-PAGE sample buffer to 5 µl supernatant and store at –20°C for SDS-PAGE analysis.**
5. **Proceed to purification protocol (page 21 or 22).**

* Benzonase® Nuclease is active in 7 M urea but inactive in 8 M urea.

Protocol: Preparation of 6xHis-tagged Periplasmic Proteins from *E. coli* Cells

Periplasmic proteins are proteins secreted into the periplasmic space located between the outer and inner membrane of *E. coli*. Proper secretion is possible only when the protein of interest has an N-terminal signal peptide which is cleaved following translocation. In order to purify proteins secreted into the periplasmic space using Ni-NTA technology, the 6xHis tag must be engineered to the C-terminus of the target protein. N-terminal 6xHis tags will be processed with the transit signal.

Materials and reagents to be supplied by user

- 30 mM Tris·Cl; 20% sucrose, pH 8.0
- 500 mM EDTA
- 5 mM $MgSO_4$
- Buffer NPI-10

Buffer compositions are provided in Appendix A on page 25.

Procedure

1. **Grow and induce a 1 liter *E. coli* culture.**
2. **Harvest the cells by centrifugation at 4,000 x *g* for 20 min. Resuspend pellet in 30 mM Tris·Cl; 20% sucrose, pH 8.0, at 80 ml per gram wet weight. Keep the cells on ice and add 500 mM EDTA dropwise to 1 mM. Incubate the cells on ice for 5–10 min with gentle agitation.**
3. **Centrifuge the cell suspension at 8000 x *g* for 20 min at 4°C, remove all the supernatant, and resuspend the pellet in the same volume of ice-cold 5 mM $MgSO_4$. Shake or stir for 10 min in an ice bath.**
4. **Centrifuge at 8000 x *g* for 20 min at 4°C.**
   The supernatant is the osmotic shock fluid containing periplasmic proteins.
5. **Dialyze supernatant extensively against lysis buffer before continuing with the purification (page 21 or 22).**

Protocol: Preparation of Cell Lysates from Insect Cells Under Native Conditions

The following procedure can be used as a starting point for developing a protocol for purification of 6xHis-tagged proteins expressed intracellularly in insect cells. However, further optimization may be necessary. Although expression rates are normally higher in insect cells than in mammalian cells, there are some difficulties connected with using baculovirus-infected insect cells for expression.

Expressed-protein levels are typically lower than those obtained in bacterial systems, and in general, smaller amounts of cell material are available. The estimated total protein content in insect cells is approximately 20 mg per 10^7 cells. With recombinant protein expression levels ranging between 0.05 % and 50% the theoretical maximum protein yield is 10 μg – 10 mg per 10^7 cells.

Buffer NPI-10 should be supplemented with 1% Igepal® CA-630 (Nonidet P40) for lysis of insect cells.

Materials and reagents to be supplied by user

- Cell pellet
- PBS, Buffer NPI-10 (supplemented with 1% Igepal CA-630 [Nonidet P40])

Buffer compositions are provided in Appendix A on page 25.

Procedure

1. **Wash the transfected cells with phosphate buffered saline (PBS) and collect them by centrifugation for 5 min at 1000 x *g*.**
2. **Lyse the cells in Buffer NPI-10 supplemented with 1% Igepal CA-630 using 4 ml buffer per 1–2 x 10^7 cells. Incubate for 10 min on ice.**

   The lysis buffer should always contain imidazole. For most 6xHis-tagged proteins, up to 20 mM imidazole can be used without affecting the binding properties. However, if the tagged protein does not bind under these conditions, the concentration of imidazole should be reduced to 5–10 mM.
3. **Centrifuge the lysate at 10,000 x *g* for 10 min at 4°C to pellet cellular debris and DNA. Save the cleared lysate (supernatant).**

   The supernatant should contain the 6xHis-tagged protein.
4. **Add 5 µl 2x SDS-PAGE sample buffer to 5 µl supernatant and store at –20°C for SDS-PAGE analysis.**
5. **Proceed to purification protocol (page 21 or 22).**

Protocol: Manual Purification of 6xHis-tagged Proteins Using a Syringe

Things to do before starting

- Immediately before applying to column, sterile filter (0.2 or 0.45 μm) or centrifuge the lysate ($\geq$ 10,000 x *g*) to ensure that it is particle-free.

Materials and equipment to be supplied by user

- Cleared cell lysate containing 6xHis-tagged protein
- Buffers NPI-10, NPI-20, and NPI-250 (native conditions) or Buffers B, C, and E (denaturing conditions). All buffers should be sterile filtered (0.2 or 0.45 μm) before use.
- 10 ml syringes
- Connector adapter

Buffer compositions are provided in Appendix A on page 25.

Procedure

1. **Fill syringe with Buffer NPI-10 (native conditions) or Buffer B (denaturing conditions), attach a suitable adapter, and expel air by depressing syringe plunger until buffer drips from the end of the adapter.**
2. **Attach syringe to the cartridge inlet and remove cartridge outlet stopper.**
3. **Equilibrate the cartridge with 10 column volumes of buffer.**
   Do not use high pressure to force buffer through the cartridge. Ideal flow rates are 1 ml/min (1 ml cartridges) and 5 ml/min (5 ml cartridges).
4. **Remove syringe and fill with cleared lysate. Apply cleared lysate to the cartridge using the same flow rate as in step 3.**
5. **Using a fresh syringe and the same flow rate as in steps 3 and 4, wash the cartridge with 10 column volumes of Buffer NPI-20 (native conditions) or Buffer C (denaturing conditions).**
6. **Fill a syringe with Buffer-NPI 250 (native conditions) or Buffer E (denaturing conditions) and elute protein from cartridge using the recommended flow rate.**
   Protein usually elutes within 5–10 column volumes.

Protocol: Purification of 6xHis-tagged Proteins Using an Automated Chromatography System

This protocol is suitable for liquid chromatography systems (such as the ÄKTAdesign™ or FPLC™ System). During equilibration, loading, washing, and elution monitor the back pressure generated by the chromatography system. Do not allow back pressure to exceed 5 bar (0.5 MPa).

Materials and equipment to be supplied by user

- Cleared cell lysate containing 6xHis-tagged protein. Immediately before applying it to the column, sterile filter (0.2 or 0.45 μm) or centrifuge the lysate ($\geq$10,000 x *g*) to ensure that it is particle-free.
- Buffers NPI-10, NPI-20, and NPI-250 (native conditions) or Buffers B, C, and E (denaturing conditions). All buffers should be sterile filtered (0.2 or 0.45 μm) before use
- Connector adapter(s)

Buffer compositions are provided in Appendix A on page 25.

Procedure

1. **Fill system pumps with Buffer NPI-10 (native conditions) or Buffer B (denaturing conditions) and attach cartridge to the pump outlet, taking care not to introduce air into the system.**
2. **Remove cartridge outlet stopper and attach to the system tubing.**
3. **Equilibrate the cartridge with 10 column volumes of buffer.**
   Use a flow rate of 1 ml/min (1 ml cartridges) or 5 ml/min (5 ml cartridges).
4. **Using the system pumps or a superloop load the cleared cell lysate onto the cartridge.**
   To ensure that the target protein has bound to the Ni-NTA resin, retain the flow-through fraction for analysis by SDS-PAGE.
5. **Fill system pumps with Buffer NPI-20 (native conditions) or Buffer C (denaturing conditions) and wash the cartridge until the A_{280} returns to the baseline value.**
   Retain the wash fraction for analysis by SDS-PAGE.
6. **Fill system pumps with Buffer-NPI 250 (native conditions) or Buffer E (denaturing conditions) and elute protein from cartridge.**
   Protein usually elutes within 5–10 column volumes. A shallow linear gradient over 20 column volumes may help separate proteins.

Troubleshooting Guide

	Comments and suggestions
Back pressure exceeds 5 bar (0.5 MPa)	
a) Column is clogged	Perform the cleaning-in-place procedure (see Appendix B). The lysate loaded onto the cartridge may have contained particles. Sterile filter (0.2 or 0.45 μm) or centrifuge prior to loading onto column.
b) High viscosity buffers used	Organic solvents or stabilizers such as glycerol can cause increased back pressure. Reduce flow rate accordingly.
Protein does not bind to the Ni-NTA Superflow resin	
a) 6xHis tag is not present	Check expression construct. Sequence ligation junctions to ensure that the reading frame is correct. Check for possible internal translation starts (N-terminal tag) or premature termination sites (C-terminal tag).
b) 6xHis tag is inaccessible	Purify protein under denaturing conditions. Move tag to the opposite end of the protein.
c) 6xHis tag has been degraded	Check that the 6xHis tag is not associated with a portion of the protein that is processed.
d) Binding conditions incorrect	Check pH of all buffers. Dissociation of urea often causes a shift in pH. The pH values should be checked immediately prior to use.
Protein elutes in the wash buffer	
a) 6xHis tag is partially hidden	Purify under denaturing conditions.
b) Buffer conditions incorrect	Check pH of denaturing wash buffer.
Protein precipitates during purification	
a) Temperature is too low	Perform purification at room temperature.

	Comments and suggestions
b) Protein forms aggregates	Try adding solubilization reagents such as 0.1% Triton® X-100 or Tween®-20, up to 20 mM β-ME, up to 2 M NaCl, or stabilizing cofactors such as Mg^{2+}. These may be necessary in all buffers to maintain protein solubility.
Protein does not elute	
a) Protein has precipitated on the cartridge	Elute under denaturing conditions.
b) Protein is still bound to the cartridge	Check pH of Denaturing Elution Buffer. Adjust to pH 4.5 if necessary.
Protein elutes with contaminants	
Contaminants are truncated forms of the tagged protein	Check for possible internal translation starts (C-terminal tag) or premature termination sites (N-terminal tag). Prevent protein degradation during purification by working at 4°C or by including protease inhibitors.

Appendix A: Buffer Compositions

NPI-10* (Binding/lysis buffer for native conditions, 1 liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
10 mM imidazole	0.68 g imidazole (MW 68.08 g/mol)

Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 µm).

* 1% Igepal CA-630 (Nonidet P40) should be added to lysis buffer when preparing cleared lysates from insect or mammalian cells.

NPI-20 (Wash buffer for native conditions, 1 Liter)

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
20 mM imidazole	1.36 g imidazole (MW 68.08 g/mol)

Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 µm).

NPI-250 (Elution buffer for native conditions, 1 Liter)

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
250 mM imidazole	17.0 g imidazole (MW 68.08 g/mol)

Adjust pH to 8.0 using NaOH and sterile filter (0.2 or 0.45 µm).

Buffer B (Denaturing lysis/binding buffer, 1 Liter)

8 M Urea	480.50 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
100 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)

Adjust pH to 8.0 using HCl and sterile filter (0.2 or 0.45 µm).

Buffer B/7 M urea (Denaturing lysis/binding buffer, 1 Liter)

7 M Urea	394.20 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
100 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)

Adjust pH to 8.0 using HCl and sterile filter (0.2 or 0.45 µm).

Buffer C (Denaturing wash buffer, 1 liter)

8 M Urea	480.50 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
100 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)

Adjust pH to 6.3 using HCl and sterile filter (0.2 or 0.45 µm).

Buffer E (Denaturing elution buffer, 1 liter)

8 M Urea	480.50 g urea (60.06 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
100 mM Tris·Cl	12.10 g Tris·Cl (MW 121.1 g/mol)

Adjust pH to 4.5 using HCl and sterile filter (0.2 or 0.45 µm).

PBS (1 liter)

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
150 mM NaCl	8.77 g NaCl (MW 58.44 g/mol)

Adjust pH to 7.2 using NaOH and sterile filter (0.2 or 0.45 µm).

2x SDS-PAGE sample buffer

0.09 M Tris·Cl, pH 6.8; 20% glycerol; 2% SDS; 0.02% bromophenol blue; 0.1 M DTT

Appendix B: Cleaning and Regeneration of Ni-NTA Superflow Cartridges

Cleaning-in-place protocol

If an increase in back pressure or significant contamination of the resin is observed, a cleaning-in-place procedure, which usually fully restores performance, can be performed. Due to the high chelating strength and the resulting low metal-leaching rate of all Ni-NTA IMAC resins, stripping is not required prior to the cleaning-in-place procedure. We recommend using the following protocol to remove contaminants such as precipitated proteins, hydrophobically bound proteins, and lipoproteins.

Procedure

1. **Wash cartridge with 15 column volumes of 0.5 M NaOH. Allow for a contact time of 30 minutes and adjust flow rate accordingly (e.g., wash a 1 ml Ni-NTA Superflow Cartridge with 0.5 M NaOH at a flow rate of 0.5 ml/min, corresponding to a total volume of 15 ml).**
2. **Re-equilibrate with 10 column volumes of Buffer NPI-10.**

   The cartridge is now ready for use. Store cartridge in 20–30% ethanol or 10–100 mM NaOH.

Regeneration by stripping and recharging

Stripping and recharging of Ni-NTA Superflow Cartridges is usually not necessary. If an increase in back pressure or significant contamination of the resin is observed, a cleaning-in-place procedure usually restores performance. However, if performance is still not satisfactory, the Ni-NTA resin in the cartridge can be stripped and recharged using the protocol below.

Procedure

1. **Strip the resin by washing with 10 column volumes of stripping buffer (50 mM Na phosphate; 300 mM NaCl; 100 mM EDTA; pH 8.0).**
2. **Wash the resin with 20 column volumes of deionized water.**
3. **Recharge the water-washed cartridge by loading 2 column volumes of 100 mM $NiSO_4$ (in deionized water). Salts of other metals (chlorides or sulfates) may also be used.**
4. **Wash with 10 column volumes of deionized water and re-equilibrate with 10 column volumes of Buffer NPI-10.**

   The cartridge is now ready for use. Store cartridge in 20–30% ethanol or 10–100 mM NaOH.

Ordering Information

Product	Contents	Cat. no.
Ni-NTA Superflow Cartridges (5 x 1 ml)	5 cartridges pre-filled with 1 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30721
Ni-NTA Superflow Cartridges (100 x 1 ml)	100 cartridges pre-filled with 1 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30725
Ni-NTA Superflow Cartridge (1 x 5 ml)	1 cartridge pre-filled with 5 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30760
Ni-NTA Superflow Cartridges (5 x 5 ml)	5 cartridges pre-filled with 5 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30761
Ni-NTA Superflow Cartridges (100 x 5 ml)	100 cartridges pre-filled with 5 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30765
Related products		
pQE vectors — for high-level expression of recombinant proteins carrying 6xHis tags		
C-Terminus pQE Vector Set	25 µg each: pQE-16, pQE-60, pQE-70	32903
N-Terminus pQE Vector Set	25 µg each: pQE-9, pQE-30, pQE-31, pQE-32, pQE-40	32915
cis-Repressed pQE Kan Vector Set	25 µg each: pQE-80L, pQE-81L, pQE-82L	32943
pQE-30 Xa Vector	25 µg pQE-30 Xa Vector DNA	33203
pQE-TriSystem Vector	25 µg pQE-TriSystem Vector DNA	33903

Product	Contents	Cat. no.
TAGZyme pQE Vector Set	TAGZyme pQE-1 and pQE-2 Vector DNA, 25 µg each	32932
***E. coli* cells — for regulated high-level expression with pQE vectors**		
E. coli Host Strains	One stab culture each: *E. coli* M15[pREP4], SG13009[pREP4]	34210
EasyXpress large-scale kits — for cell-free synthesis of recombinant proteins for structural studies		
EasyXpress NMR Protein Synthesis Kit	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o Arg, Lys, Ser, Thr, Val (supplied as individual amino acids), RNase-free water, gel-filtration columns, and reaction flasks	32526
EasyXpress NMR Protein Synthesis Kit – X	For 2 x 5 ml reactions: *E. coli* extract, reaction buffers, amino acid mix w/o amino acid X (to be supplied by user), RNase-free water, gel-filtration columns, and reaction flasks	Varies
Anti·His antibodies and conjugates — for sensitive and specific detection of 6xHis-tagged proteins		
RGS·His Antibody (100 µg)	100 µg mouse anti-RGS(His)$_4$ (lyophilized, with BSA, for 1000 ml working solution)	34610
RGS·His Antibody, BSA-free, (100 µg)	100 µg mouse anti-RGS(His)$_4$ BSA-free (lyophilized, for 1000 ml working solution)	34650
Penta·His Antibody, BSA-free (100 µg)	100 µg mouse anti-(His)$_5$ (lyophilized, BSA-free, for 1000 ml working solution)	34660
Tetra·His Antibody, BSA-free (100 µg)	100 µg mouse anti-(His)$_4$ (lyophilized, BSA-free, for 1000 ml working solution)	34670
Anti·His Antibody Selector Kit	RGS·His Antibody, Penta·His Antibody, Tetra·His Antibody, all BSA-free, 3 µg each	34698
RGS·His HRP Conjugate Kit	125 µl RGS·His HRP Conjugate, 5 g Blocking Reagent, 50 ml Blocking Reagent Buffer, 10x Concentrate	34450
Penta·His HRP Conjugate Kit	125 µl Penta·His HRP Conjugate, 5 g Blocking Reagent, 50 ml Blocking Reagent Buffer, 10x Concentrate	34460

Product	Contents	Cat. no.
6xHis Protein Ladder	6xHis-tagged marker proteins (lyophilized, for 50–100 lanes on western blots)	34705
6xHis-tag removal systems		
TAGZyme Kit	For processing of approximately 10 mg tagged protein: 0.5 units DAPase Enzyme, 30 units Qcyclase Enzyme, 10 units pGAPase Enzyme, 20 mM Cysteamine·HCl (1 ml), Ni-NTA Agarose (10 ml), 20 Disposable Columns	34300
Factor Xa Protease	400 units Factor Xa Protease (2 units/µl)	33223
Xa Removal Resin	2 x 2.5 ml Xa Removal Resin, 3 x 1.9 ml 1 M Tris·Cl, pH 8.0	33213

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Trademarks: QIAGEN®, QIA*express*® (QIAGEN Group); ÄKTAdesign™, FPLC™ (Amersham plc); Benzonase® (Merck KGaA, Germany); Igepal® (Rhone Poulenc, Inc.); Triton® (Rohm & Haas, Inc.); Tween® (ICI Americas, Inc.)

Benzonase® Nuclease is supplied by Merck KGaA and its Affiliates. Benzonase® is a registered trademark of Merck KGaA, Darmstadt, Germany.

Hoffmann-La Roche owns patents and patent applications pertaining to the application of Ni-NTA resin (in certain countries) and to 6xHis-coding vectors and His-labeled proteins. All purification of recombinant proteins by Ni-NTA chromatography for commercial purposes, and the commercial use of proteins so purified, require a license from Hoffmann-La Roche in certain countries.

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the Ni-NTA Superflow Cartridge to the following terms:

1. The Ni-NTA Superflow Cartridge may be used solely in accordance with the *Ni-NTA Superflow Cartridge Handbook* and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the *Ni-NTA Superflow Cartridge Handbook* and additional protocols available at www.qiagen.com.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

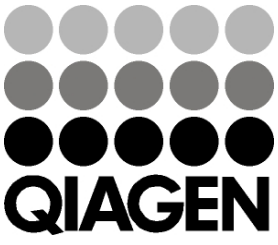
Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)



1069564 07/2011

Sample & Assay Technologies