Second Editon

January 2008

Ni-NTA Spin Kit Handbook

Ni-NTA Spin Kit

Ni-NTA Spin Columns

For manual or automated purification of His-tagged proteins

QIAGEN®

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QIAGEN Sample and Assay Technologies

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- Purification of DNA, RNA, and proteins
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- microRNA research and RNAi
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Contents

Kit Contents

	Ni-NTA Spin Columns	Ni-NTA Spin Kit
Catalog No.	**31014**	**31314**
Ni-NTA Spin Columns	50	50
2 ml Collection Microtubes	50	50
Guanidine HCl		40 g
Urea		100 g
1 M Imidazole, pH 7.0		50 ml
5x Phosphate Buffer Stock Solution (0.5 M NaH_2PO_4; 50 mM Tris·Cl, pH 8.0)		100 ml
Control Vector DNA		1 µg

Storage Conditions

Ni-NTA Spin Kits and Ni-NTA Spin Columns should be stored at 2–8°C. They can be stored under these conditions for up to 18 months without any reduction in performance.

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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding Ni-NTA Spin Columns or Kits 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 see our Technical Support center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Product Use Limitations

QIA*express*® products are developed, designed, and sold for research use. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

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 back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Ni-NTA spin columns and kits is tested against predetermined specifications to ensure consistent product quality.

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.

Buffer A used for protein purification under denaturing conditions contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

CAUTION: DO NOT add bleach or acidic solutions directly to Buffer A.

If these buffers are spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to the components of Ni-NTA Spin Kits.

Ni-NTA Spin Columns

Contains nickel-nitrilotriacetic acid. Risk and safety phrases*: R22-40-42/43. S13-26-36-46

Sodium phosphate stock solution, 5x

Contains sodium hydroxide: Irritant. Risk and safety phrases*: R36/38. S13-26-36-46

Guanidine hydrochloride

Contains guanidine hydrochloride: Harmful, Irritant. Risk and safety phrases*: R22-36/38. S22-26-36/37/39

Imidazole solution

Contains imidazole: Irritant. Risk and safety phrases*: R36/37/38. S23-26-36/37/39-45

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

* R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R36/37/38: Irritating to eyes, respiratory system and skin; R40: Possible risks of irreversible effects; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S22: Do not breathe dust; S23: Do not breathe vapor. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible); S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

Ni-NTA Spin Kits provide a simple method for rapid screening and purification of 6xHis-tagged proteins from small-scale expression cultures. Proteins can be purified using either a manual procedure or a fully automated procedure on the QIAcube®.

This protein purification system is based on the remarkable selectivity of our unique Ni-NTA resin for recombinant proteins carrying a small affinity tag consisting of 6 consecutive histidine residues, the 6xHis tag. Ni-NTA Spin Kits provide all the advantages of QIA*express* Ni-NTA protein affinity purification (please refer to *The QIA*expressionist™) in a convenient microspin format.

Ni-NTA Spin Kits are based on Ni-NTA Silica, a unique and versatile metal chelate chromatography material, packaged in ready-to-use spin columns. They allow rapid purification of proteins from crude cell lysates under either native or denaturing conditions. The one-step procedure allows purification of up to 300 µg 6xHis-tagged protein per column in as little as 15 minutes.

General Information

The high affinity of the Ni-NTA resins for 6xHis-tagged proteins or peptides is due to both the specificity of the interaction between histidine residues and immobilized nickel ions and to the strength with which these ions are held to the NTA resin. QIA*express* nickel-chelating resin utilizes our unique, patented NTA (nitrilotriacetic acid) ligand. NTA has a tetradentate chelating group that occupies four of six sites in the nickel coordination sphere. The metal is bound much more tightly than to a tridentate chelator such as IDA (imidodiacetic acid), which means that nickel ions — and as a result the proteins — are very strongly bound to the resin. This allows more stringent washing conditions, better separation, higher purity, and higher capacity — without nickel leaching.

Ni-NTA Silica combines all of the benefits of Ni-NTA with a silica material that has been modified to provide a hydrophilic surface. Nonspecific hydrophobic interactions are kept to a minimum, while the silica support allows efficient microspin technology. Ni-NTA spin columns are supplied precharged with nickel ions, ready for use.

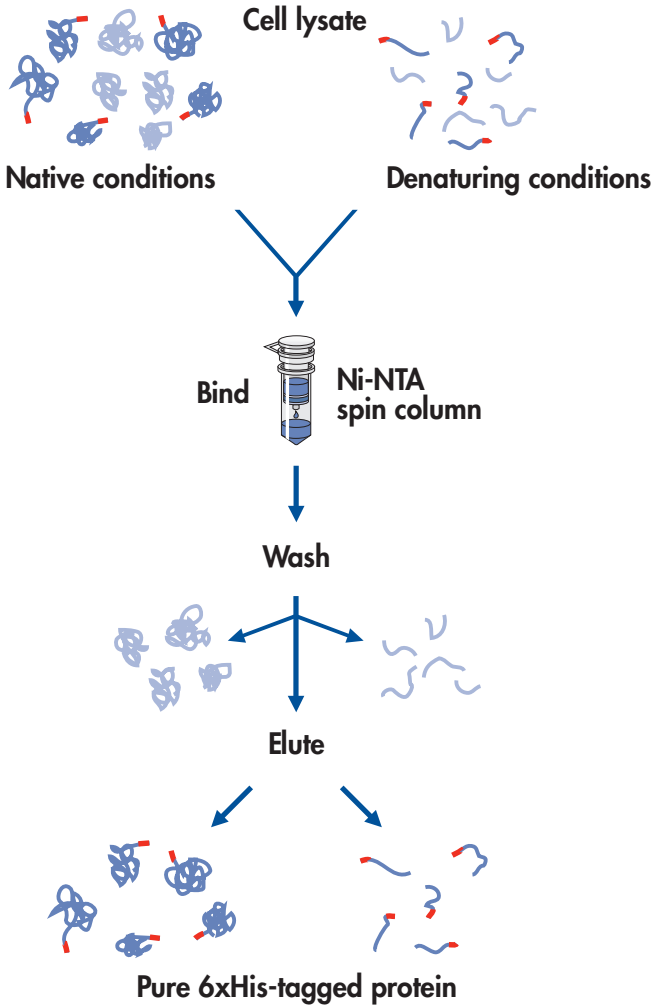

Figure 1. Ni-NTA Spin purification procedure.

Automated purification

Purification of His-tagged proteins can be fully automated on the QIAcube®. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using the Ni-NTA Spin Kit for purification of high-quality His-tagged proteins. Purification of recombinant proteins on the QIAcube starts with resuspension of cell pellets and proceeds until purified proteins are eluted from the resin. For more information about the automated procedure, see the relevant protocol sheet available at www.qiagen.com/MyQIAcube .

Figure 2. The QIAcube.

The QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube .

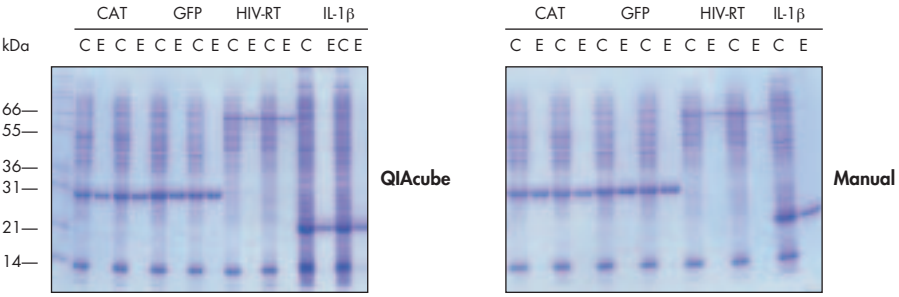

Figure 3. Efficient automated and manual purification of His-tagged proteins using Ni-NTA spin columns. The indicated proteins were purified in duplicate under native conditions using Ni-NTA Spin Columns from cleared *E. coli* cell lysates derived from 5 ml LB cultures either manually or in an automated procedure on the QIAcube. **CAT**: chloramphenicol acetyl transferase; **GFP**: Green fluorescent protein; **HIV-RT**: Human immunodeficiency virus reverse transcriptase; **IL-1**β: Interleukin-1 beta. **M**: markers; **C**: cleared lysate (2 µl loaded per lane); **E**: elution fraction (3 µl loaded per lane).

Denaturing or Native Purification — Protein Solubility and Cellular Location

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 renaturation 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, nontagged 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. One of the denaturing buffers listed in Appendix B will completely solubilize most 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. Inclusion bodies of some membrane proteins may not be solubilized under chaotropic conditions, but may be able to be solubilized using detergents.

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 column itself prior to elution, or in solution; suggestions can be found in *The QIA*expressionist.

Protocols for purification of His-tagged proteins from *E. coli* lysates under both denaturing and native conditions are available for the QIAcube. Visit www.qiagen.com/MyQIAcube for more details.

Table 1. Compatibility of reagents with Ni-NTA

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 or phosphate-citrate 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
Nonionic detergents (β-OG, DM, DDM, Cymal 6, Apo12 9, NG and others)	Resolubilization and purification of membrane proteins	Up to 2% can be used
Zwitterionic detergents (LDAO, CHAPS, CHAPSO)	Removal of background proteins and nucleic acids; purification of membrane proteins	Up to 1% can be used

Reagent	Effect	Comments
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
Denaturants		
GuHCl	Solubilize proteins	Up to 6 M
Urea		Up to 8 M
Amino acids		
Glycine		Not recommended
Glutamine		Not recommended
Glutamic acid		Up to 100 mM
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 nonspecific 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
$LiCl_2$		Up to 50 mM
$CaCl_2$		Up to 5 mM
$MgSO_4$		Up to 1 M
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
Triethanolamine	Prevents hydrophobic interactions	Up to 100 mM

Reagent	Effect	Comments
Sorbitol, betaine, ectoine	Prevents hydrophobic interactions	Up to 500 mM
Dextran sulfate	Prevents hydrophobic interactions	Up to 0.05% (w/v)
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

Purification of 6xHis-tagged Proteins — Ni-NTA Spin Procedure

The Ni-NTA silica in the spin columns has the same purification properties and elution profile as Ni-NTA Agarose and is compatible with the buffer systems used for large-scale protein purification with Ni-NTA Agarose. Although the Ni-NTA Spin Kit procedure has been designed for the purification of 6xHis-tagged proteins from bacterial expression systems, the system can also be used for the purification of 6xHis-tagged recombinant proteins expressed in other hosts. The procedure will work very well for most 6xHis-tagged proteins, but some modifications may be necessary if an expression system other than *E. coli* is used (see *The QIA*expressionist for details). A protocol for purification of His-tagged proteins from baculovirus-infected insect cells is available for the QIAcube. New protocols are constantly being developed. For an up-to-date list, visit www.qiagen.com/MyQIAcube .

General considerations and limitations

- To ensure efficient binding, it is important not to exceed 270 x *g* (approx. 1600 rpm) when centrifuging Ni-NTA spin columns. At higher forces, even if the binding kinetics are high, the time the lysate is in contact with the resin is not sufficient for effective binding.
- Since silica is not inert in solutions of high pH, buffers with pH >8.4 should not be used with the Ni-NTA silica material.
- Avoid high concentrations of buffer components containing strong electron-donating groups (see Table 1).
- Cells should be lysed without the use of strong chelating agents such as EDTA or ionic detergents (e.g., SDS). Although low levels of these reagents have been used successfully, leaching may occur, and performance may be diminished (see Table 1, page 11).
- Please take into account that the time needed for the centrifugation step during protein binding is influenced by the viscosity of the cleared lysate. For very concentrated cell lysates, it may be necessary to extend the centrifugation time to 5 or 10 min at 270 x *g* (approx. 1600 rpm).
- The spin columns should be centrifuged with an open lid to ensure that the centrifugation step is completed after 2 min. Under native conditions, it may be preferable to centrifuge with a closed lid to reduce the flow rate thereby extending binding time.

- Some proteins may be subject to degradation during cell harvest, lysis, or even during growth after induction. In these cases, addition of PMSF (0.1–1 mM) or other protease inhibitors into the growth medium may be considered. PMSF treatment during cell growth may result, however, in lower expression levels. Under native conditions it is best to work quickly and at 4°C at all times and use protease inhibitors in all buffers.

Microspin procedure summary

The purification procedure can be divided into three stages:

- Preparation of the cell lysate and binding of the 6xHis-tagged protein to Ni-NTA silica
- Washing
- Elution of the 6xHis-tagged protein

Up to 600 µl of cell lysate is loaded onto a Ni-NTA spin column and centrifuged for 5 minutes to bind 6xHis-tagged proteins to the Ni-NTA silica. Most of the nontagged proteins flow through. Residual contaminants and nontagged proteins are removed by washing with buffers of slightly reduced pH or with buffers containing a low concentration of imidazole. Purified protein is eluted in a volume of 100–300 µl using acidic pH values or high concentrations (>100 mM) of imidazole.

Preparation of the cell lysate and protein binding under denaturing conditions

Cells can be lysed in either 6 M GuHCl or 8 M urea. It is preferable to lyse the cells in the milder denaturant, urea, so that the cell lysate can be analyzed directly on an SDS polyacrylamide gel. GuHCl is a more efficient solubilization and cell lysis reagent, however, and may be required to solubilize some proteins. Since fractions which contain GuHCl will precipitate with SDS when loaded onto an SDS polyacrylamide gel, they must either be diluted (1:6), dialyzed before analysis, or separated from GuHCl by TCA precipitation (see Appendix A, page 29). Some membrane proteins may not be solubilized under chaotropic conditions, but may be able to be solubilized using detergents.

It is important to estimate the expression level of your protein, for example using SDS-PAGE. For proteins that are expressed at very high levels (>10 mg per liter assuming 10^9 bacterial cells per ml, i.e., equivalent to an expression level of >12% of total cellular protein), the cell lysate needs to be concentrated 10-fold relative to the original culture volume. The pellet of a 10-ml culture, for example, can be lysed in 1 ml lysis buffer. For an expression level of 10 mg per liter, 600 µl of the 10x cell lysate in Buffer B would contain approximately 60 µg of 6xHis-tagged protein.

For lower expression levels (2–5 mg/liter) 25x cell lysates (600 µl cell lysate = 30–75 µg) should be prepared for loading onto the Ni-NTA spin column. If expression levels are expected to be lower than 1 mg per liter, the cell lysate should be prepared at a 50-fold concentration.

For control purposes, mouse dihydrofolate reductase (DHFR) can be expressed. Using the supplied expression plasmid, DHFR can be expressed in any *Escherichia coli* strain with the *lacI*^{q} mutation. DHFR is expressed at 40 mg/liter.

Preparation of the cell lysate and protein binding under native conditions

Before purifying proteins under native conditions, it is important to determine how much of the protein is soluble in the cytoplasm and how much is in insoluble precipitates or inclusion bodies. Parallel purification under denaturing conditions is recommended.

Because of variations in protein structure that can interfere with binding, it is difficult to provide an exact protocol for purification of tagged proteins under native conditions. However, some general guidelines are helpful to optimize the purification procedure:

- Since there is often a higher background under native conditions, low concentrations of imidazole in the lysis and washing buffers are recommended. Binding of nontagged contaminating proteins is inhibited, leading to greater purity in fewer steps.
- For most proteins, up to 10–20 mM imidazole can be used without affecting the yield. However, if the tagged protein does not bind under these conditions, the amount of imidazole should be reduced to 1–5 mM.
- Addition of β-mercaptoethanol (up to 20 mM) or DTT (up to 10 mM) reduces any disulfide bonds which may have formed between contaminating proteins and the 6xHis-tagged protein. Under some circumstances, however, especially when the proteins have a strongly reducing character, nickel leaching may occur or the resin may turn brown. This does not usually compromise purity or quality of the purified protein.
- Cell pellets frozen for at least 30 minutes at –20°C can be lysed by resuspending in lysis buffer and addition of lysozyme (1 mg/ml) and Benzonase® Nuclease (3 Units/ml culture volume). Fresh, (i.e., unfrozen) pellets require sonication or homogenization in addition to the lysozyme/Benzonase® treatment. Detergent-based lysis buffer formulations may also be used, but are usually less efficient.
- All buffers should have sufficient ionic strength to prevent nonspecific interactions between proteins and the Ni-NTA matrix. The minimum salt concentration during binding and washing steps should be 300 mM NaCl. The maximal concentration is 2 M NaCl.

- For control purposes, mouse dihydrofolate reductase (DHFR) can be expressed using the control vector DNA supplied with the Ni-NTA Spin Kit in any *E. coli* strain with the *lacI*^{q} mutation. The DHFR protein is expressed at 40 mg/liter in *E. coli* M15 [pREP4] after 4 hours of induction at 37°C. Only 10% of DHFR will present in the cells in a soluble form; the remainder can be solubilized and purified under denaturing conditions.

Protein elution

Elution of the tagged proteins from the column can be achieved either by reducing the pH, or by competition with imidazole. Monomers are generally eluted at approximately pH 5.9 or with imidazole concentrations greater than 100 mM, whereas multimers elute at around pH 4.5 or 200 mM imidazole. Elution using Buffer E (pH 4.5) or buffers containing 250–500 mM imidazole at pH 8 (e.g., Buffer NPI-500) is therefore recommended. 100 mM EDTA elutes all bound protein by stripping nickel from the resin.

Using a Ni-NTA spin column, up to 300 µg of 6xHis-tagged protein can be purified to up to 90% homogeneity. Actual yields and purity will vary depending on the size and expression level of the recombinant protein, as well as the viscosity of the lysate. The recommended elution volume is 200–300 µl. To obtain even higher protein concentrations, elution volumes can be reduced to 100–200 µl.

Protocol: Growth of Expression Cultures

Materials and equipment to be supplied by user*

- LB medium: 10 g/liter bacto-tryptone, 5 g/liter bacto yeast extract, and 5 g/liter NaCl
- Kanamycin stock solution: 10 mg/ml in water, sterilize by filtration, store at –20°C. Use at a final concentration of 25 µg/ml (i.e., dilute 1 in 400).
- Ampicillin stock solution: 100 mg/ml in water, sterilize by filtration, store at –20°C. Use at a final concentration of 100 µg/ml (i.e., dilute 1 in 1000).
- IPTG stock solution: 1 M IPTG (e.g., QIAGEN cat. no. 129921) in water, sterilize by filtration, store at –20°C

Protocol

1. **Inoculate 10 ml of LB medium containing the appropriate antibiotics with a fresh bacterial colony harboring the expression plasmid. Grow at 37°C overnight.**

   The antibiotics kanamycin and ampicillin are appropriate for *E. coli* M15[pREP4] or SG13009[pREP4] harboring pQE expression vectors. Other host strain–vector combinations may require selection with different antibiotics.

2. **Dilute the non-induced overnight culture 1:60 (e.g., inoculate 30 ml medium with 500 µl overnight culture) with fresh LB medium containing the appropriate antibiotics. Grow at 37°C with vigorous shaking until the OD_{600} reaches 0.6.**

   For control purposes, mouse dihydrofolate reductase (DHFR) can be expressed using the control vector DNA supplied with the Ni-NTA Spin Kit in any *E. coli* strain with the *lacI*^{q} mutation. The DHFR protein is expressed at 40 mg/liter in *E. coli* M15 [pREP4] after 4 hours of induction at 37°C. Only 10% of DHFR will present in the cells in a soluble form; the remainder can be solubilized and purified under denaturing conditions.

   The required volume of expression culture is mainly determined by the expression level, solubility of the protein, and purification conditions. For purification of proteins expressed at low levels, especially under native conditions, the minimum cell culture volume should be 50 ml.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

3. **Add IPTG to a final concentration of 1 mM and grow the culture at 37°C with vigorous shaking for 4 hours.**

   For proteins which are very sensitive to protein degradation, the induction time should be reduced and a time course of expression should be determined. In some cases, addition of 0.1–1 mM PMSF after induction is recommended to inhibit PMSF sensitive proteases. PMSF treatment can result, however, in a lower expression level.

4. **Harvest the cells by centrifugation at 4000 x *g* for 15 min.**

   Store cell pellet at –20°C if desired or process immediately as described for purification under denaturing conditions (page 20) or for purification under native conditions (page 23).

Protocol: Protein Purification under Denaturing Conditions from *E. coli* Lysates

Materials and equipment to be supplied by user

- Buffer A*: 6 M GuHCl; 0.1 M NaH_2PO_4; 0.01 M Tris·Cl; pH 8.0
- Buffer B - 7M urea: 7 M urea; 0.1 M NaH_2PO_4; 0.01 M Tris·Cl; pH 8.0
- Buffer C: 8 M urea; 0.1 M NaH_2PO_4; 0.01 M Tris·Cl; pH 6.3
- Buffer D*: 8 M urea; 0.1 M NaH_2PO_4; 0.01 M Tris·HCl; pH 5.9
- Buffer E: 8 M urea; 0.1 M NaH_2PO_4; 0.01 M Tris·Cl; pH 4.5
- Benzonase® Endonuclease 25 U/µl (e.g., Novagen, cat. no. 70664-3)

Due to the dissociation of urea, the pH values of Buffers B, C, D, and E should be checked and, if necessary, adjusted immediately prior to use. Do not autoclave.

This protocol is suitable for use with frozen cell pellets. Cell pellets frozen for at least 30 minutes at –20°C can be lysed by resuspending in lysis buffer and adding Benzonase® Nuclease (3 Units/ml culture volume). Fresh (i.e., not frozen) pellets require sonication or homogenization in addition to the addition of 3 Units/ml culture volume Benzonase® Nuclease and 1 mg/ml culture volume lysozyme.

Protocol

1. **Thaw cells for 15 min and resuspend in 700 µl Buffer B – 7 M urea and add 3 Units/ml culture volume Benzonase® Nuclease (i.e., for cell pellets from 5 ml cultures, add 15 Units Benzonase® Nuclease).**

   Cells from 5 ml cultures are usually used, but culture volume used depends on protein expression level. Resuspending pellet in 700 µl buffer will allow recovery of a volume of cleared lysate of approx. 600 µl.

2. **Incubate cells with agitation for 15 min at room temperature.**

   The solution should become translucent when lysis is complete. Buffer B is the preferred lysis buffer, as the cell lysate can be analyzed directly by SDS-PAGE. If the cells or the protein do not solubilize in Buffer B, then Buffer A must be used. Since fractions which contain GuHCl will precipitate with SDS when loaded onto an SDS polyacrylamide gel, they must either be diluted (1:6), dialyzed before analysis, or separated from GuHCl by TCA precipitation (see Appendix A, page 29). Please note that Benzonase® is inactive in the presence of GuHCl concentrations >100 mM. If cells are lysed in GuHCl, genomic DNA must be sedimented by centrifugation during collection of the cleared lysate supernatant, as aggregated gDNA may clog the Ni-NTA spin column.

* Buffer A and D are not necessary for all proteins

3. **Centrifuge lysate at 12,000 x *g* for 15–30 min at room temperature (20–25°C) to pellet the cellular debris. Collect supernatant.**

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

4. **Equilibrate a Ni-NTA spin column with 600 µl Buffer B – 7 M urea. Centrifuge for 2 min at 890 x *g* (approx. 2900 rpm).**

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

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

   For proteins which are expressed at very high expression levels (50–60 mg of 6xHis-tagged protein per liter of cell culture) a 3x–5x concentrated cell lysate can be used. 600 µl of a 5x concentrated cell lysate in Buffer B will contain approximately 150–180 µg of 6xHis-tagged protein. For lower expression levels (1–5 mg/liter), 50 ml of cell culture should be used, to give a 50x concentrated cell lysate (600 µl cell lysate = 30–150 µg) of 6xHis-tagged protein.

   To ensure efficient binding, it is important not to exceed 270 x *g* (approx. 1600 rpm) when centrifuging Ni-NTA spin columns. At higher forces, even if the binding kinetics are high, the time the lysate is in contact with the resin is not sufficient for effective binding.

   Please take into account that the time needed for the centrifugation step during protein binding is influenced by the viscosity of the cleared lysate. For very concentrated cell lysates, it may be necessary to extend the centrifugation time to 3 or 4 min at 700 x *g* (approx. 2000 rpm).

   Save the flow-through for analysis by SDS-PAGE to check binding.

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

   This wash step can be carried out with Buffer C even if Buffer A was used to initially solubilize the protein. Most proteins will remain soluble in Buffer C. If this is not the case, Buffer C and Buffer E should be made with 6 M guanidine hydrochloride instead of 8 M urea.

   Save the flow-through (wash fractions) for analysis by SDS-PAGE to check the stringency of the wash conditions.

7. **Repeat step 6.**

   It may not be necessary to wash twice with Buffer C. The number of wash steps required to obtain highly pure protein is determined primarily by the expression level of the 6xHis-tagged protein. When the expression level is high, 2 wash steps are usually sufficient for removal of contaminants. For very low expression levels or highly concentrated lysates, 3 wash steps may be required to achieve high purity.

Save the flow-through (wash fractions) for analysis by SDS-PAGE to check the stringency of the wash conditions.

8. **Elute the protein twice with 200 µl Buffer E. Centrifuge for 2 min at 890 x *g* (approx. 2900 rpm), and collect the eluate.**

   Most of the 6xHis-tagged protein (>80%) should elute in the first 200 µl, especially when proteins smaller than 30 kDa are purified. The remainder will elute in the second 200 µl. If higher protein concentrations are desired, do not combine the eluates or, alternatively, elute in 100–150 µl aliquots.

   If 6xHis-tagged monomers need to be separated from multimers, an elution step using Buffer D may be performed before elution with Buffer E.

Protocol: Protein Purification under Native Conditions from *E. coli* Lysates

Materials and equipment to be supplied by user*

- Lysis Buffer (NPI-10): 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0
- Wash Buffer (NPI-20): 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0
- Elution Buffer (NPI-500): 50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8.0
- Benzonase® Endonuclease 25 U/µl (e.g., Novagen cat. no. 70664-3)
- Lysozyme (e.g., Roche cat. no. 837059) stock solution 10 mg/ml in water. Sterilize by filtration and store in aliquots at –20°C.

Note: The 5x Phosphate Buffer Solution supplied with the Ni-NTA Spin Kit contains Tris and should not be used to prepare buffers for purification under native conditions.

Protocol

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).**

   Cells from 5 ml cultures are usually used, but culture volume used depends on protein expression level. Resuspending pellet in 700 µl buffer will allow recovery of a volume of cleared lysate of approx. 600 µl.

   Do not use pellets from culture volumes greater than 70 ml. If larger culture volumes are processed, resuspend in 1.4 ml and load the supernatant in 2 successive portions of 600 µl in step 5. By adding 10 mM imidazole, binding of nontagged contaminating proteins is inhibited, leading to greater purity in fewer steps. If the tagged protein does not bind under these conditions, the amount of imidazole should be reduced to 1–5 mM.

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.**

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

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

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

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

   By adding 10 mM imidazole, the binding of nontagged contaminating proteins is inhibited, leading to greater purity in fewer steps. If the tagged protein does not bind under these conditions the amount of imidazole should be reduced to 1–5 mM.

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.**

   To ensure efficient binding it is important not to exceed 270 x *g* (approx. 1600 rpm) when centrifuging Ni-NTA spin columns. At higher forces, even if the binding kinetics are high, the time the lysate is in contact with the resin is not sufficient for effective binding.

   The spin columns can be centrifuged with an open lid to ensure that the centrifugation step is completed after 5 min, but under native conditions, it may be preferable to centrifuge with a closed lid to reduce the flow rate thereby extending binding time.

   Please take into account that the time needed for the centrifugation step during protein binding is influenced by the viscosity of the cleared lysate. For very concentrated cell lysates, it may be necessary to extend the centrifugation time to 5 or 10 min at 270 x *g* (approx. 1600 rpm).

   Save the flow-through for analysis by SDS-PAGE to check binding efficiency.

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).**

   The number of wash steps required to obtain highly pure protein is determined primarily by the expression level of the 6xHis-tagged protein. When the expression level is high, 2 washes are usually sufficient for removal of contaminants. For very low expression levels or highly concentrated lysates, 3 wash steps may be required to achieve high purity.

   Save the flow-through (wash fractions) for analysis by SDS-PAGE to check the stringency of the wash conditions.

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.**

   Most of the 6xHis-tagged protein (>80%) should elute in the first 300 µl eluate. The remainder will elute in the second 300 µl. If higher protein concentrations are desired, do not combine the eluates or, alternatively, elute in 100–200 µl aliquots.

Protocol: Protein Purification under Native Conditions from Insect Cell Lysates

Materials and equipment to be supplied by user*

- PBS: 50 mM potassium phosphate; 150 mM NaCl, pH 7.2
- Lysis Buffer (NPI-10-Ig): 50 mM NaH_2PO_4; 300 mM NaCl; 10 mM imidazole; 1% Igepal® CA-630, pH 8.0
- Wash Buffer (NPI-20): 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0
- Elution Buffer (NPI-500): 50 mM NaH_2PO_4, 300 mM NaCl, 500 mM imidazole, pH 8.0
- Benzonase® Endonuclease 25 U/µl (e.g., Novagen, cat. no. 70664-3)

Note: The 5x Phosphate Buffer Solution supplied with the Ni-NTA Spin Kit contains Tris and should not be used to prepare buffers for purification under native conditions.

Protocol

1. **Wash 5 x 10^6 transfected cells with PBS and collect by centrifugation at 1000 x *g* for 5 min.**
2. **Resuspend the cell pellet in 700 µl Lysis Buffer (NPI-10-Ig). Add 3 Units/ml culture volume Benzonase® Nuclease (i.e., for cell pellets from 5 x 10^6 cells, add 15 Units Benzonase® Nuclease).**

   By adding 10 mM imidazole to the lysis buffer, the binding of nontagged contaminating proteins is inhibited, leading to greater purity in fewer steps. If the tagged protein does not bind under these conditions the amount of imidazole should be reduced to 1–5 mM.
3. **Incubate on ice for 15–30 min.**
4. **Centrifuge lysate at 12,000 x *g* for 15–30 min at 4°C. Collect supernatant.**

   Save 20 µl of the cleared lysate for SDS-PAGE analysis.
5. **Equilibrate the Ni-NTA spin column with 600 µl Lysis Buffer (NPI-10-Ig). Centrifuge for 2 min at 890 x *g* (approx. 2900 rpm).**

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

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

6. **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.**

   To ensure efficient binding it is important not to exceed 270 x *g* (approx. 1600 rpm) when centrifuging Ni-NTA spin columns. At higher forces, even if the binding kinetics are high, the time the lysate is in contact with the resin is not sufficient for effective binding.

   The spin columns can be centrifuged with an open lid to ensure that the centrifugation step is completed after 5 min, but under native conditions, it may be preferable to centrifuge with a closed lid to reduce the flow rate thereby extending binding time.

   Please take into account that the time needed for the centrifugation step during protein binding is influenced by the viscosity of the cleared lysate. For very concentrated cell lysates, it may be necessary to extend the centrifugation time to 5 or 10 min at 270 x *g* (approx. 1600 rpm).

   Save the flow-through for analysis by SDS-PAGE to check binding efficiency.

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

   The number of wash steps required to obtain highly pure protein is determined primarily by the expression level of the 6xHis-tagged protein. When the expression level is high, 2 washes are usually sufficient for removal of contaminants. For very low expression levels or highly concentrated lysates, 3 wash steps may be required to achieve high purity.

   Save the flow-through (wash fractions) for analysis by SDS-PAGE to check the stringency of the wash conditions.

8. **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.**

   Most of the 6xHis-tagged protein (>80%) should elute in the first 300 µl eluate. The remainder will elute in the second 300 µl. If higher protein concentrations are desired, do not combine the eluates or, alternatively, elute in 100–200 µl aliquots.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Protein does not bind to the Ni-NTA Spin column		
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. Ensure that there are no chelating or reducing agents present and that the concentration of imidazole is not too high (see Table 1, page 11).
Protein elutes in the wash buffer		
a)	Wash stringency is too high	Lower the concentration of imidazole or increase the pH slightly.
b)	6xHis tag is partially hidden	Purify under denaturing conditions.
c)	Buffer conditions incorrect	Check pH of denaturing wash buffer.

	Comments and suggestions
Protein precipitates during purification	
a) Temperature is too low	Perform purification at room temperature.
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	
Elution conditions are too mild (protein may be in an aggregate or multimer form)	Elute with decreased pH or increased imidazole concentration.
Protein elutes with contaminants	
a) Binding and wash conditions not stringent enough	Include 10–20 mM imidazole in the binding and wash buffers
b) Contaminants are associated with tagged protein	Add β-mercaptoethanol to a maximum of 20 mM to reduce disulfide bonds. Increase salt and/or detergent concentrations, or add ethanol/glycerol to wash buffer to disrupt nonspecific interactions (see Table 1, page 11).
c) 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: Preparation of Guanidine-Containing Samples for SDS-PAGE

Since the fractions that contain GuHCl will form a precipitate when treated with SDS, they must either be diluted with water (1:6), dialyzed before analysis, or separated from the guanidine hydrochloride by trichloroacetic acid (TCA) precipitation.

TCA-precipitation: Bring the volume of the samples up to 100 µl with water, add an equal volume of 10% TCA, leave on ice 20 min, spin 15 min at 15,000 x *g* in a microcentrifuge, wash pellet with 100 µl of ice-cold ethanol, dry, and resuspend in 1x SDS-PAGE sample buffer (5x SDS-PAGE sample buffer is 0.225 M Tris·Cl, pH 6.8; 50% glycerol; 5% SDS; 0.05% bromophenol blue; 0.25 M DTT). In case there is still any GuHCl present, samples should be loaded immediately after boiling for 7 min at 95°C.

Appendix B: Buffer Compositions

Bacterial media and solutions

LB medium	10 g/liter tryptone; 5 g/liter yeast extract; 10 g/liter NaCl
LB agar	LB medium containing 15 g/liter agar
Psi broth	LB medium; 4 mM $MgSO_4$; 10 mM KCl
Kanamycin stock solution	25 mg/ml in H_2O, sterile filter, store in aliquots at –20°C
Ampicillin stock solution	100 mg/ml in H_2O, sterile filter, store in aliquots at –20°C
IPTG (1 M)	238 mg/ml in H_2O, sterile filter, store in aliquots at –20°C

Buffers for purification under native conditions

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).

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-500 (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)
500 mM imidazole	34.0 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.

Buffers for purification under denaturing conditions

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

6 M GuHCl	573 g guanidine hydrochloride (95.53 g/mol)
100 mM NaH_2PO_4	13.80 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
10 mM Tris·Cl	1.21 g Tris base (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 base (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 base (MW 121.1 g/mol)

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

Buffer D (Denaturing elution buffer for separation of monomeric proteins, 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 base (MW 121.1 g/mol)

Adjust pH to 5.9 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 base (MW 121.1 g/mol)

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

Ordering Information

Product	Contents	Cat. no.
Ni-NTA Spin Kit (50)	50 Ni-NTA Spin Columns, Reagents, Buffers, Collection Tubes, 1 µg Control Expression Plasmid	31314
Ni-NTA Spin Columns (50)	50 Ni-NTA Spin Columns, Collection Tubes	31014
Related products		
Ni-NTA Agarose (25 ml)*	25 ml nickel-charged resin (max. pressure: 2.8 psi)	30210
Ni-NTA Superflow (25 ml)*	25 ml nickel-charged resin (max. pressure: 140 psi)	30410
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 5 ml)	100 cartridges pre-filled with 5 ml Ni-NTA Superflow: for automated purification of His-tagged proteins using liquid chromatography systems	30765
Expression vectors — for high-level expression of recombinant proteins carrying 6xHis tags		
QIAgenes Expression Kit	QIAgenes Expression Construct (10 µg), QIAgenes *E. coli* Positive Control (10 µg), Penta·His Antibody, BSA-free (3 µg), 4 Ni-NTA Spin Columns	Please inquire
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 Vector Set	25 µg each: pQE-80L, pQE-81L, pQE-82L	32923
pQE-100 DoubleTag	25 µg pQE-100 (lyophilized) Vector DNA	33003

* Other pack sizes and bulk quantities available; please inquire.

Ordering Information

Product	Contents	Cat. no.
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pQE-TriSystem Vector	25 µg pQE-TriSystem Vector DNA	33903
QIAexpress UA Cloning Kit	100 µl 2x Ligation Master Mix, 1 µg pQE-30 UA Vector DNA (50 ng/µl), distilled water	32179
His·*Strep* pQE-TriSystem Vector Set	pQE-TriSystem His·*Strep 1* and pQE-TriSystem His·*Strep 2* vectors, 25 µg each	32942
TAGZyme pQE Vector Set	TAGZyme pQE-1 and pQE-2 Vector DNA, 25 µg each	32932
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EasyXpress kits — for cell-free synthesis of recombinant proteins		
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EasyXpress Protein Synthesis Mini Kit	For 20 x 50 µl reactions: *E. coli* extract, reaction buffers, RNase-free water, and positive-control DNA	32502
Anti·His antibodies and conjugates — for sensitive and specific detection of 6xHis-tagged proteins		
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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

Ordering Information

Product	Contents	Cat. no.
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
Tetra·His HRP Conjugate Kit	125 µl Tetra·His HRP Conjugate, 5 g Blocking Reagent, 50 ml Blocking Reagent Buffer, 10x Concentrate	34470
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

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Hoffmann-La Roche owns patents and patent applications pertaining to the application of Ni-NTA resin (Patent series: RAN 4100/63: USP 4.877.830, USP 5.047.513, EP 253 303 B1), and to 6xHis-coding vectors and His-labeled proteins (Patent series: USP 5.284.933, USP 5.130.663, EP 282 042 B1). 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.

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