Ni-NTA Spin Handbook

For
Ni-NTA Spin Kit
Ni-NTA Spin Columns

February 2003

QIAGEN

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Contents

Kit Contents

Ni-NTA Spin Kits **Catalog No.**	**(50)** **31314**
Ni-NTA Spin Columns	50
2 ml Collection Microtubes	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 molecular biology and the use of QIAGEN® products. If you have any questions or experience any difficulties regarding Ni-NTA Spin Kits, or QIAGEN products in general, please do not hesitate to contact us.

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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 bacterial expression cultures. 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 (Figure 1). The one-step procedure allows purification of up to 150 µg 6xHis-tagged protein in just 20 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.

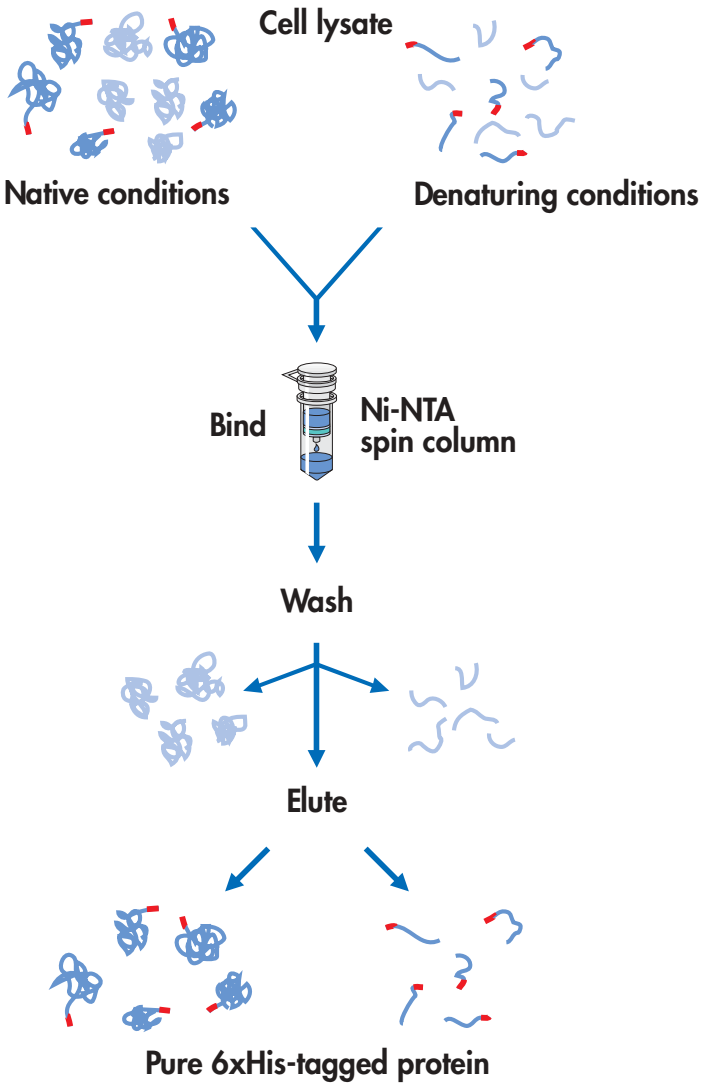

Figure 1. *Ni-NTA Spin purification procedure.*

Denaturing or Native Purification — Protein Solubility and Cellular Location

Since the interaction between Ni-NTA and the 6xHis tag of the recombinant protein does not depend on tertiary structure, proteins can be purified either under denaturing or native conditions (Figure 1). To develop the best purification strategy, it is important to determine whether the protein is soluble in the cytoplasm or located in inclusion bodies. Many proteins form inclusion bodies when they are expressed at high levels in bacteria. If the protein is found in inclusion bodies, it must be solubilized with strong denaturants, such as guanidine hydrochloride (GuHCl) or urea, prior to purification on Ni-NTA. Purification under denaturing conditions ensures that all 6xHis-tagged protein in the cell is solubilized and can be purified. In addition, the 6xHis tag is fully exposed under denaturing conditions which leads to more efficient purification.

If purification under native conditions is preferred or necessary, the 6xHis-tagged protein must be soluble under these conditions. Purification under non-denaturing conditions can result in reduced yields if the 6xHis tag is only partly exposed due to protein folding. However, even when most of the protein is present in inclusion bodies, there may be some soluble material which can be purified in its native form.

Purification of 6xHis-tagged proteins under native conditions may allow copurification of associated proteins such as enzyme subunits and binding proteins (Figure 2). Under native conditions however, there is a higher potential for unrelated, nontagged proteins to interact with the resin than under denaturing conditions. This is reflected in the larger number of proteins that appear in the first wash. Nonspecific binding can be reduced by including a low concentration of imidazole in the lysis and wash buffers.

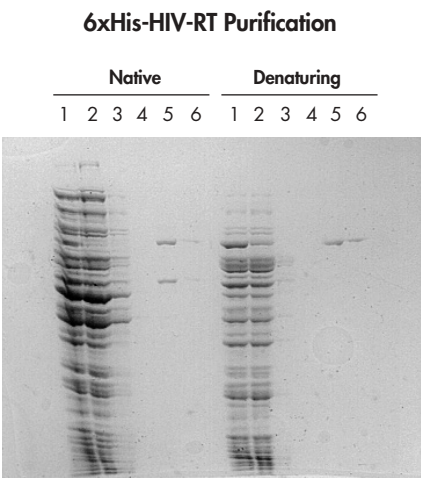

Figure 2. *Following expression in* E. coli, *6xHis-tagged HIV reverse transcriptase was purified using the Ni-NTA Spin Kit under native (lysis and wash at 20 mM imidazole; elution at 200 mM imidazole) or denaturing conditions (8 M urea; elution at pH 5.9). Under native conditions, the small untagged subunit of HIV-RT is copurified. 3.5 µl of each 200 µl eluate were loaded. Proteins were visualized by Coomassie® staining.* ***1:*** *cell lysate;* ***2:*** *flow-through;* ***3 & 4:*** *washes;* ***5 & 6:*** *eluates.*

Table 1. Compatibility of reagents with Ni-NTA matrices

Reagent	Effect	Comments
Buffer reagents		
Tris, HEPES, MOPS	• Buffers with secondary or tertiary amines will reduce nickel ions	• Up to 100 mM has been used successfully in some cases
		• 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	• Up to 20 mM can be used
	• Can reduce nickel ions at higher concentration	
DTT, DTE	• Low concentrations will reduce nickel ions	• A maximum of 1 mM may be used, but β-mercaptoethanol is recommended
Detergents		
Nonionic detergents (Triton®, Tween®, NP-40, etc.)	• Remove 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
Denaturant		
GuHCl	• Solubilize proteins	• Up to 6 M
Urea		• Up to 8 M

Reagent	Effect	Comments
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%
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

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).

General considerations and limitations

- It is important not to exceed 700 x *g* (approx. 2000 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 (e.g., glycine, arginine, Tris; see Table 1).
- Cells should be lysed without the use of strong chelating agents such as EDTA, strong reducing agents such as DTT, or ionic detergents (e.g., SDS). Although low levels of these reagents have been used successfully, leaching may occur, and performance may be diminished.
- The concentration of the cell lysate should be adjusted according to the expression level (see Table 2).
- 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).
- 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 is recommended. 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.

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, and elution of the 6xHis-tagged protein. Up to 600 µl of cell lysate is loaded onto a Ni-NTA spin column and centrifuged for 2 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 subsequently eluted in aliquots of 100–200 µl. A total of 400 µl is sufficient to quantitatively elute the bound protein.

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.

It is important to estimate the expression level of your protein, for example using SDS-PAGE (Figure 3). 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 must only be concentrated 10-fold relative to the original culture volume. The pellet of a 10-ml culture, for example, should 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 (see page 16) would contain approximately 60 µg of 6xHis-tagged protein (see Table 2).

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 (see Table 2).

For control purposes, mouse dihydrofolate reductase (DHFR) can be expressed in any *Escherichia coli* strain with the *lacI*^{q} mutation. DHFR is expressed at 40 mg/liter *E. coli* culture after 4 hours of induction.

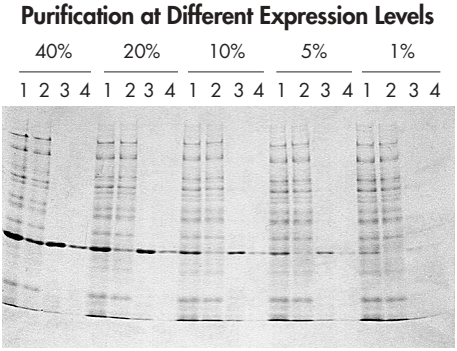

Figure 3. *6xHis-tagged mouse DHFR was expressed at the indicated levels in* E. coli*, purified from 3-ml cultures using the Ni-NTA Spin Kit under denaturing conditions, and eluted in buffer at pH 5.9. Fractions were visualized by Coomassie staining after SDS-PAGE. 5 µl of each 200 µl eluate were loaded.* ***1:*** *cell lysate;* ***2:*** *flow-through* ***3:*** *first eluate;* ***4:*** *second eluate.*

Table 2. Recommended culture volumes based on expression level and purification conditions for a cell lysis volume of 1 ml

Concentration of 6xHis-tagged protein	Expression level	Culture volume	6xHis-tagged protein in 600 µl lysate	Concentration factor
Denaturing Conditions				
50 mg/liter	40%	3 ml	90 µg	3x
10 mg/liter	8%	10 ml	60 µg	10x
2 mg/liter	1.6%	25 ml	30 µg	25x
0.5 mg/liter	0.4%	50 ml	15 µg	50x
0.1 mg/liter	0.08%	100 ml	6 µg	100x
Native Conditions				
>1 mg/liter	>1%	50 ml	>30 µg	50x
<1 mg/liter	<1%	100 ml	<60 µg	100x

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) 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.
- Cells can be lysed by sonication or homogenization after treatment with lysozyme (1 mg/ml). Cells and buffers should be kept at 0–4°C at all times. The addition of protease inhibitors may also be necessary.
- 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 in any *Escherichia coli* strain with the *lacI*^{q} mutation. DHFR is expressed at 40 mg/liter *E. coli* culture after 4 hours of induction. Only 10% of DHFR is present in the cells in soluble form, and will be solubilized using native 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 mM imidazole (pH 8) is therefore recommended. 100 mM EDTA elutes all bound protein as a protein–metal complex.

Table 3. Concentration of 6xHis-tagged DHFR as a function of elution volume

Eluate	Elution volume (µl)	Yield (µg)	Concentration (µg/µl)
1	200	118	0.59
2	200	20	0.10
1+2	400	138	0.35
1	150	101	0.67
2	150	35	0.23
1+2	300	136	0.45
1	100	78	0.78
2	100	34	0.34
1+2	200	112	0.56

Using a Ni-NTA spin column up to 150 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. To obtain even higher protein concentrations, elution volumes can be reduced, as shown in Table 3.

Protocol: Growth of Expression Cultures

Buffers and reagents

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
Ampicillin stock solution:	100 mg/ml in water, sterilize by filtration, store at –20°C
IPTG stock solution:	1 M IPTG (238.3 mg/ml) in water, sterilize by filtration, store at –20°C

Protocol

1. **Inoculate 10 ml of LB medium containing 100 µg/ml ampicillin and 25 µg/ml kanamycin 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; see Table 2 for culture volume required for purification) 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 in any *E. coli* strain with the *lacI*^{q} mutation. The DHFR protein is expressed at 40 mg/liter after 4 hours of induction. Only 10% of DHFR is present in the cells in soluble form, the remainder will not be solubilized using native 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 (see Table 2).

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.**

5. **Store cell pellet at –70°C if desired or process immediately as described for purification under denaturing conditions (next section) or for purification under native conditions (page 20).**

Protocol: Protein Purification under Denaturing Conditions

Buffers and reagents

Buffer A*: 6 M GuHCl; 0.1 M NaH_2PO_4; 0.01 M Tris·Cl; pH 8.0

Buffer B: 8 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·Cl; pH 5.9

Buffer E: 8 M urea; 0.1 M NaH_2PO_4; 0.01 M Tris·Cl; pH 4.5

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.

Protocol

1. **Thaw cells for 15 min and resuspend in 1 ml Buffer B.**

   Cells from 10 ml culture are usually used, but it depends on the expression level (see Table 2).

2. **Incubate cells with agitation for 1 h at room temperature.**

   The solution should become translucent when lysis is complete. It is preferable to lyse the cells in Buffer B so that 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 upon loading onto an SDS polyacrylamide gel, they must either be diluted (1:6), dialyzed before analysis, or separated from GuHCl by TCA precipitation (see Appendix, page 23).

3. **Centrifuge lysate at 10,000 x *g* for 20–30 min at RT (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. Centrifuge for 2 min at 700 x *g* (approx. 2000 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 2 min at 700 x *g* (approx. 2000 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

** Buffers A and D are not necessary for all proteins*

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 (see also Table 2).

It is important not to exceed 700 x *g* (approx. 2000 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 700 x *g* (approx. 2000 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, Buffer D, 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 700 x *g* (approx. 2000 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 dilution of the protein is undesirable, do not combine the eluates or, alternatively, elute in 100–150 µl aliquots (see Table 3).

Protocol: Protein Purification under Native Conditions

Buffers and reagents*

Lysis Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0

Wash Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0

Elution Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0

Lysozyme

Protocol

1. **Thaw cells for 15 min and resuspend in 1 ml Lysis Buffer.**

   For purification under native conditions, the minimum cell culture volume processed should be 50 ml resulting in a 50x concentrated cell lysate.

   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. **Add lysozyme to 1 mg/ml.**
3. **Incubate on ice for 30 min.**
4. **Sonicate or homogenize on ice to lyse cells (six times for 10 s each time with 5 s pauses between).**
5. **Centrifuge lysate at 10,000 x *g* for 20–30 min at 4°C. Collect supernatant.**

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

6. **Equilibrate the Ni-NTA spin column with 600 µl Lysis Buffer. Centrifuge for 2 min at 700 x *g* (approx. 2000 rpm).**

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

7. **Load up to 600 µl of the cleared lysate containing the 6xHis-tagged protein onto the pre-equilibrated Ni-NTA spin column. Centrifuge for 2 min at 700 x *g* (approx. 2000 rpm), and collect the flow-through.**

   To increase the yield of the 6xHis-tagged protein 50x concentrated cell lysates should be used, especially when expression levels are low (see Table 2).

   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.

** The 5x Phosphate Buffer Stock Solution supplied with the Ni-NTA Spin Kit contains 50 mM Tris and should not be used to prepare buffers for protein purification under native conditions.*

It is important not to exceed 700 x *g* (approx. 2000 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 2 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 3 or 4 min at 700 x *g* (approx. 2000 rpm).

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

8. Wash the Ni-NTA spin column twice with 600 µl Wash Buffer. Centrifuge for 2 min at 700 x *g* (approx. 2000 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.

9. Elute the protein twice with 200 µl Elution Buffer. Centrifuge for 2 min at 700 x *g* (approx. 2000 rpm), and collect the eluate.

Most of the 6xHis-tagged protein (>70%) should elute in the first 200 µl eluate. The remainder will elute in the second 200 µl. If dilution of the protein is undesirable, do not combine the eluates or, alternatively, elute in 100–150 µl aliquots (see Table 3).

Troubleshooting Guide

	Comments and suggestions
Protein does not bind to the Ni-NTA spin column	
a) 6xHis tag is not present	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 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 and composition of all buffers and solutions. Dissociation of urea often causes a shift in pH. Check pH values immediately before use. Ensure that there are no chelating or reducing agents present, and that the concentration of imidazole is not too high (see Table 1).
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	Reduce wash stringency. Purify under denaturing conditions.
c) Buffer conditions incorrect	Check pH and composition of the wash buffer. Ensure that there are no chelating or reducing agents present.
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-mercaptoethanol, up to 2 M NaCl, or stabilizing cofactors, such as Mg^{2+}. These may be necessary in all buffers to maintain protein solubility.

	Comments and suggestions
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).
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: 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.

Ordering Information

Product	Contents	Cat. No.
Ni-NTA Agarose		
Ni-NTA Agarose (25 ml)	25 ml nickel-charged resin (max. pressure: 2.8 psi)	30210
Ni-NTA Agarose (100 ml)	100 ml nickel-charged resin (max. pressure: 2.8 psi)	30230
Ni-NTA Agarose (500 ml)	500 ml nickel-charged resin (max. pressure: 2.8 psi)	30250
Ni-NTA Superflow		
Ni-NTA Superflow (25 ml)	25 ml nickel-charged resin (max. pressure: 140 psi)	30410
Ni-NTA Superflow (100 ml)	100 ml nickel-charged resin (max. pressure: 140 psi)	30430
Ni-NTA Superflow (500 ml)	500 ml nickel-charged resin (max. pressure: 140 psi)	30450
Ni-NTA Spin Columns		
Ni-NTA Spin Columns (50)	50 Spin Columns, Collection Tubes	31014
Ni-NTA Spin Kit		
Ni-NTA Spin Kit (50)	50 Ni-NTA Spin Columns, Reagents, Buffers, Collection Tubes, 1 µg Control Expression Plasmid	31314
QIA*express* Kits		
QIA*express* Type IV Kit	5 µg each of pQE-30, pQE-31, pQE-32 (N-terminal 6xHis), 10 ml NiNTA Agarose*	32149
QIA*express* Type ATG Kit	5 µg each of pQE-60 and pQE-70 (C-terminal 6xHis), 10 ml Ni-NTA Agarose*	32169

** Also included in QIAexpress Kits: 1 µg pREP4, 1 µg Control Expression Plasmid, E. coli host strains M15[pREP4] and SG13009[pREP4], 5 x 1ml bed-volume and 5 x 5 ml bed-volume disposable plastic columns, reagents, and a comprehensive handbook.*

Ordering Information

Product	Contents	Cat. No.
QIA*express* Expression Systems		
C-Terminus pQE Vector Set	25 µg each of pQE-16, pQE-60, and pQE-70	32903
N-Terminus pQE Vector Set	25 µg each of pQE-9, pQE-30, pQE-31, pQE-32, and pQE-40	32915
cis-Repressed pQE Vector Set	25 µg each: pQE-80L, pQE-81L, and pQE-82L	32923
pQE-100 DoubleTag Vector DNA	25 µg of pQE-100 (lyophilized)	33003
pQE Sequencing-Primer Set	0.1 A_{260} unit each: Primer - Promoter Region, Primer - Type III/IV, Primer - Reverse Sequencing (3.0, 2.8, 3.1 µg, respectively; lyophilized)	34051
E. coli Host Strains	One stab culture each of *E. coli* M15[pREP4] and SG13009[pREP4]	34210
QIA*express* Detection Systems		
Ni-NTA AP Conjugate	Alkaline-phosphatase–conjugated Ni-NTA (lyophilized, for 500 ml working solution)	34510
Ni-NTA HRP Conjugate	Horseradish-peroxidase–conjugated Ni-NTA (lyophilized, for 500 ml working solution)	34530
RGS·His Antibody (100 µg)	100 µg mouse anti-RGS$(H)_4$ antibody (lyophilized, with BSA, for 1000 ml working solution)	34610
RGS·His Antibody, BSA-free (100 µg)	100 µg mouse anti-RGS$(H)_4$ antibody (lyophilized, BSA-free, for 1000 ml working solution)	34650
Penta·His Antibody, BSA-free (100 µg)	100 µg mouse anti-$(H)_5$ antibody (lyophilized, BSA-free, for 1000 ml working solution)	34660
Tetra·His Antibody, BSA-free (100 µg)	100 µg mouse anti-$(H)_4$ antibody (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
Tag·100 Antibody, BSA-free (100 µg)	100 µg mouse anti-Tag·100 antibody (lyophilized, BSA-free, for 1000–2500 ml working solution for ELISA)	34680

Please inquire for availability and pricing of bulk resins and antibody.

Ordering Information

Product	Contents	Cat. No.
Ni-NTA HisSorb Strips and Plates		
Ni-NTA HisSorb Strips (24)	2 racks of 12 x Ni-NTA–coated 8-well strips in 96-well format	35023
Ni-NTA HisSorb Plates (5)	5 Ni-NTA–coated, transparent 96-well plates	35061
Ni-NTA HisSorb Plates, white (5)	5 Ni-NTA–coated, opaque, white 96-well plates	35081
Ni-NTA Magnetic Agarose Beads		
Ni-NTA Magnetic Agarose Beads (2 x 1 ml)	2 x 1 ml nickel-charged magnetic agarose beads (5% suspension)	36111
Ni-NTA Magnetic Agarose Beads (6 x 1 ml)	6 x 1 ml nickel-charged magnetic agarose beads (5% suspension)	36113
12-Tube Magnet	Magnet for separating magnetic beads in 1.5-ml or 2-ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic beads in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells, 24 per case, for use with the 96-Well Magnet	36985

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