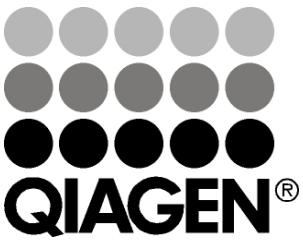
July 2011

QIA*express*® Ni-NTA Fast Start Handbook

For purification and detection of recombinant 6xHis-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

Ni-NTA Fast Start Kit (6) **For six 6xHis-tagged protein preparations**	**Cat. no. 30600**
Lysis Buffer Native, pH 8.0	1 x 60 ml
Wash Buffer Native, pH 8.0	1 x 60 ml
Elution Buffer Native, pH 8.0	1 x 30 ml
Denaturing Buffer	1 x 150 ml
Fast Start Columns (0.5 ml)	6
Benzonase® Nuclease	1 x 80 µl (25 U/µl)
Penta·His Antibody, BSA-Free	1 x 3 µg
Lysozyme	1 x 60 mg
Handbook	1

Storage and Stability

Fast Start Columns and Native Lysis, Wash, and Elution Buffers should be stored at 2–8°C. Denaturing Buffer should be stored at 2–8°C. Benzonase® Nuclease and Lysozyme should be stored at –20°C.

Penta·His Antibodies should be stored lyophilized until they are to be used. They can be stored lyophilized for 1 year at 2–8°C. In solution they can be stored for 3 months at 2–8°C or for up to 6 months in aliquots at –20°C. Avoid repeated freezing and thawing. Dissolve Penta·His Antibody (3 µg) in 15 µl water per tube (final concentration, 0.2 mg/ml).

Product Use Limitations

The Ni-NTA Fast Start Kit 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.

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).

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 the Ni-NTA Fast Start Kit 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).

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 the components of the Ni-NTA Fast Start Kit:

Penta·His Antibody, BSA-Free

Contains anti-His antibody. Sensitizer. Risk and safety phrases*: R42/43. S24-26-36/37.

Fast Start Columns

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. S24: Avoid contact with skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36: Wear suitable protective clothing. S36/37: Wear suitable protective clothing and gloves. S46: If swallowed, seek medical advice immediately and show the container or label.

Introduction

Producing a recombinant version of a given protein greatly simplifies its purification and detailed characterization and offers many advantages.

Large amounts of protein

Expression of recombinant proteins in *E. coli* can enable production of gram amounts of protein from relatively small amounts of biomass. This is in contrast to purifying proteins from native sources, where large amounts of starting material may be required to yield small amounts of pure protein. In addition, most purification procedures for non-recombinant proteins require multiple steps, in each of which a proportion of the target protein is lost.

Addition of affinity tags

Addition of an affinity tag enables any protein to be obtained at a high level of purity using a single, standardized affinity chromatography procedure. Such purification procedures avoid the losses inherent to multistep procedures and eliminate the need to develop a protein-specific purification procedure for each new target protein. Addition of an affinity tag can also facilitate detection of recombinant proteins. Antibodies that react specifically with the affinity tag epitope can be used as a universal detection reagent, sparing the time and expense of generating protein-specific antibodies.

Generation of mutants

Once the coding sequence of a protein is cloned into an expression vector, molecular biological methods enable mutations to be made in the sequence, easily allowing substitution or deletion of amino acids in structure–function studies.

Workflow for Producing Recombinant Proteins

The process of producing recombinant proteins in *E. coli* cells can be broken down into the following steps:

- Cloning the protein sequence into an expression plasmid, transforming *E. coli* cells, and selecting and growing cells containing the expression plasmid
- Inducing expression and harvesting of cells
- Purification of recombinant protein
- Analysis of protein expression and the purification procedure

This handbook provides a concise overview of the techniques and principles involved in producing recombinant proteins. More detailed information and additional protocols can be found in *The QIA*expressionist®, the *QIA*express *Detection and Assay Handbook*, and the QIAGEN *Bench Guide*. All are available to view and download in a convenient PDF format at www.qiagen.com.

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.

Cloning

In order to express a recombinant protein, its coding DNA sequence must be cloned into an expression vector. The QIA*express* system offers several different expression vectors, many of which offer special features for specific applications (see Table 2).

Table 2. QIA*express* System Expression Vectors and their Properties

Application	Vector(s)
Expression of proteins with an N-terminal 6xHis-tag	
Standard expression	pQE-9, -30, and -80L series
Vectors for specialized applications	
For efficient 6xHis-tag removal	TAGZyme™ pQE vectors, pQE-30 Xa
For very toxic proteins	pQE-80L series
For small proteins and peptides	pQE-40
Expression of proteins with a C-terminal 6xHis-tag	
Standard expression	pQE-60, -70
Vectors for specialized applications	
For parallel expression in *E. coli*, insect, and mammalian cells	pQE-TriSystem
For small proteins and peptides	pQE-16

Introducing Expression Vectors into *E. coli* Cells

Once a protein's coding sequence has been cloned into an expression vector, the expression vector must be introduced into competent *E. coli* cells in a process termed transformation. Transformation can be performed by heat-shocking the cells (see protocol, page 13). Another commonly used method for transformation is electroporation. If using this method, consult your electroporator documentation for a suitable protocol. After transformation, an aliquot of the transformed cells is spread out onto an agar plate to allow isolation of an *E. coli* colony containing the expression plasmid. pQE expression vectors carry a gene for ampicillin resistance, which allows *E. coli* containing the expression plasmid to be selected for on agar plates containing ampicillin.

Expressing Recombinant Protein in *E. coli* Cells

Efficient and controlled expression of protein in *E. coli* cells is regulated by the presence of the *lac* repressor protein. During normal cell growth, this protein binds to the operator sequences in the plasmid and prevents recombinant protein expression. Expression of recombinant proteins encoded by pQE vectors is rapidly induced by the addition of isopropyl-β-D-thiogalactoside (IPTG) which binds to the *lac* repressor protein and inactivates it. Once the *lac* repressor is inactivated, the host cell's RNA polymerase can transcribe the sequences downstream from the promoter. The transcripts produced are then translated into the recombinant protein. Inducing protein expression with IPTG means that the cellular metabolism concentrates almost exclusively on the production of recombinant protein (see Figure 1). Tight control of expression also has the advantage that proteins that may have adverse effects on growing cells are not expressed, allowing cells to develop and grow normally until protein expression is induced.

There are two alternative methods for ensuring that enough *lac* repressor protein is present in the cells.

The *E. coli* host strains available from QIAGEN — M15[pREP4] and SG13009[pREP4] — contain multiple copies of the pREP4 plasmid which constitutively expresses the *lac* repressor protein and also confers kanamycin resistance. After transformation, *E. coli* cells containing both plasmids are selected for using a combination of kanamycin and ampicillin.

Expression vectors in the pQE-80 series contain the *lacI*^{q} gene which expresses *lac* repressor protein independent of IPTG induction. Using pQE-80 series vectors allows expression in any *E. coli* strain, and eliminates the need for a second plasmid (such as pREP4) that expresses the *lac* repressor protein. Using pQE-80L, pQE-81L, or pQE-82L with the *cis-lacI*^{q} gene, expression rates are comparable with those obtained using other pQE vectors in combination with pREP4.

Expressing Recombinant Proteins

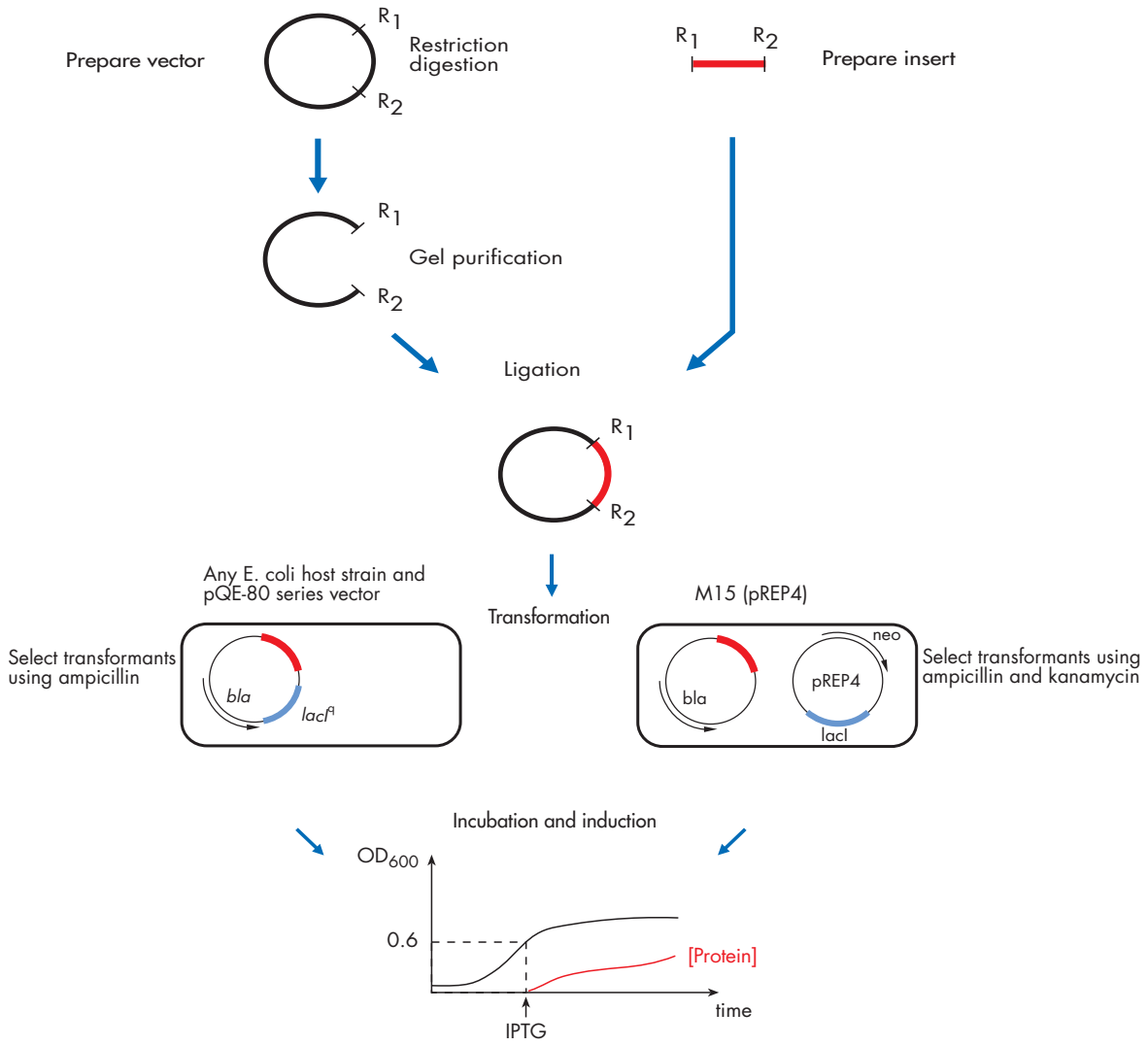


Figure 1 Steps involved in cloning an insert and expressing protein in *E. coli*.

Protocol: Preparation of Competent *E. coli* Cells

Materials to be supplied by user

- *E. coli* cells
- LB medium
- LB agar plates containing appropriate antibiotic (kanamycin if using *E. coli* cells containing the pREP4 plasmid)
- TFB1
- TFB2
- Antibiotic stock solution (kanamycin if using *E. coli* cells containing the pREP4 plasmid)

For composition of media and solutions, see appendix, page 34.

Procedure

1. **Remove a trace of *E. coli* cells from their storage vial with a sterile toothpick or inoculating loop, and streak it out on LB agar containing the appropriate antibiotic (for pREP4 use kanamycin [25 µg/ml]).**
2. **Incubate at 37°C overnight.**
3. **Pick a single colony and inoculate 10 ml of LB medium containing the appropriate antibiotic (for pREP4 use kanamycin [25 µg/ml]). Grow overnight at 37°C.**
4. **Add 1 ml overnight culture to 100 ml prewarmed LB medium containing the appropriate antibiotic (for pREP4 use kanamycin [25 µg/ml]) in a 250 ml flask, and shake at 37°C until an OD_{600} of 0.5 is reached (approximately 90–120 min).**
5. **Cool the culture on ice for 5 min, and transfer the culture to a sterile, round-bottom centrifuge tube.**
6. **Collect the cells by centrifugation at low speed (5 min, 4000 x *g*, 4°C).**
7. **Discard the supernatant carefully. Always keep the cells on ice.**
8. **Resuspend the cells gently in cold (4°C) TFB1 buffer (30 ml for a 100 ml culture) and keep the suspension on ice for an additional 90 min.**
9. **Collect the cells by centrifugation (5 min, 4000 x *g*, 4°C).**
10. **Discard the supernatant carefully. Always keep the cells on ice.**
11. **Resuspend the cells carefully in 4 ml ice-cold TFB2 buffer.**
12. **Prepare aliquots of 100–200 µl in sterile microcentrifuge tubes and freeze in liquid nitrogen or a dry-ice–ethanol mix. Store the competent cells at –70°C.**

Protocol: Transformation of Competent *E. coli* Cells

Materials and equipment to be supplied by user

- Competent *E. coli* cells
- Ligation mix (i.e., pQE expression plasmid DNA in solution)
- LB agar plates containing 100 µg/ml ampicillin (and 25 µg/ml kanamycin if using *E. coli* cells containing the pREP4 plasmid)
- Psi broth
- Heating block or water bath set to 42°C

For composition of media and solutions, see appendix, page 34.

Procedure

1. **Transfer an aliquot of the ligation mix (10 µl or less) into a cold sterile 1.5 ml microcentrifuge tube, and keep it on ice.**
2. **Thaw an aliquot of frozen competent *E. coli* cells on ice.**
3. **Gently resuspend the cells and transfer 100 µl of the cell suspension into the microcentrifuge tube with the ligation mix, mix carefully, and keep it on ice for 20 min.**
4. **Transfer the tube to a 42°C water bath or heating block for 90 sec.**
5. **Add 500 µl Psi broth to the cells and incubate for 60–90 min at 37°C.**
   Shaking increases transformation efficiency.
6. **Plate out 50, 100, and 200 µl aliquots on LB-agar plates containing 100 µg/ml ampicillin (and 25 µg/ml kanamycin if using pREP4 containing cells). Incubate the plates at 37°C overnight.**

Positive control to check transformation efficiency:

Transform competent cells with 1 ng of the pQE-40 control plasmid (undigested) in 20 µl of TE. Plate 1/100 and 1/10 dilutions of the transformation mix (diluted in prewarmed Psi broth) as well as undiluted transformation mix on LB-agar plates containing 100 µg/ml ampicillin (and 25 µg/ml kanamycin if using pREP4 containing cells). The cells should yield 10^6 transformants per microgram of plasmid.

Negative control to check antibiotic activity:

Transform cells with 20 µl of TE. Plate at least 200 µl of the transformation mix on a single LB agar plate containing the appropriate antibiotics.

Protocol: Growing *E. coli* Cells and Inducing Expression

Materials and equipment to be supplied by user

- *E. coli* cells containing expression plasmid
- LB medium
- Ampicillin and kanamycin stock solution
- IPTG stock solution (1 M)
- 5x SDS-PAGE sample buffer

For composition of media and solutions, see appendix, page 34.

Procedure

1. **Using a sterile toothpick or inoculating loop, pick a single colony from a selective antibiotic LB agar plate and inoculate 10 ml of LB medium containing 100 µg/ml ampicillin (and 25 µg/ml kanamycin if using pREP4 containing cells) in a 50 ml flask. Grow the cultures at 37°C overnight.**
2. **Inoculate 250 ml of prewarmed media (with ampicillin) with 10 ml of the overnight cultures and grow at 37°C with vigorous shaking until an OD_{600} of 0.6 is reached (30–60 min).**
3. **Take a 0.5 ml sample immediately before induction.**

   This sample is the noninduced control; pellet cells and resuspend them in 25 µl 5x SDS-PAGE sample buffer. Freeze and store the sample at –20°C until SDS-PAGE analysis.
4. **Induce expression by adding IPTG to a final concentration of 1 mM.**
5. **Incubate the cultures for an additional 4–5 h. Collect a second 0.5 ml sample.**

   This sample is the induced control; pellet cells and resuspend them in 50 µl 5x SDS-PAGE sample buffer. Freeze and store the sample at –20°C until SDS-PAGE analysis.
6. **Harvest the cells by centrifugation at 4000 x *g* for 20 min.**
7. **Freeze and store cell pellet overnight at –20°C.**

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, 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.

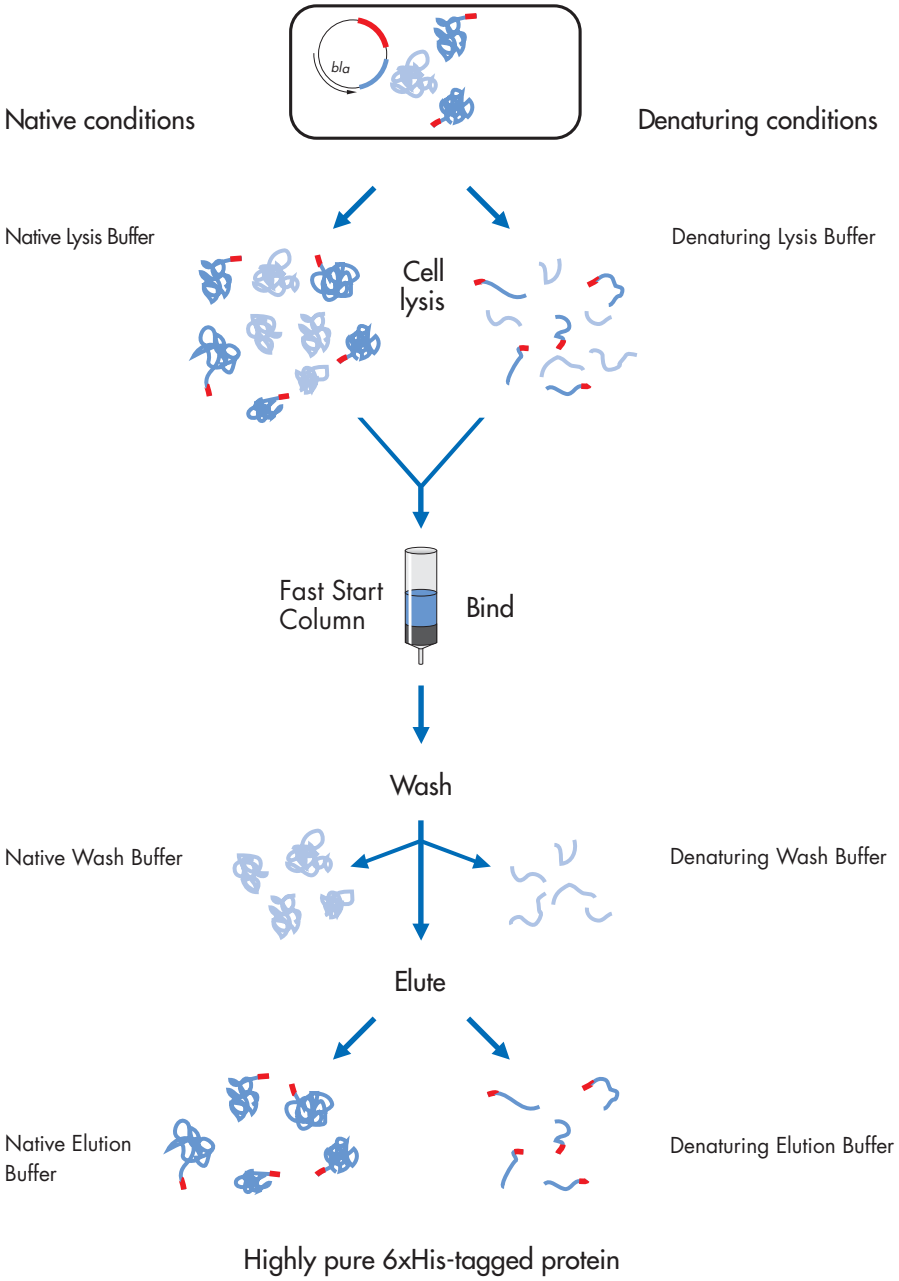
In rare cases the 6xHis tag is hidden by the tertiary structure of the native protein, so that soluble proteins require denaturation before they can be purified on Ni-NTA. As a control, a parallel purification under denaturing conditions should always be carried out: If purification is only possible 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. The Denaturing Buffer in the Ni-NTA Fast Start Kit 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 column itself prior to elution, or in solution; suggestions can be found in *The QIA*expressionist.

Purifying 6xHis-tagged Recombinant Proteins



Protocol: Purification of 6xHis-tagged Proteins Under Native Conditions

Things to do before starting

Before use, native Lysis Buffer must be supplemented with lysozyme and Benzonase® Nuclease. Dissolve the contents of the lysozyme vial in 600 µl of native Lysis Buffer. Add 100 µl of the lysozyme solution to a 10 ml aliquot of native Lysis Buffer. Store the remaining lysozyme solution at –20°C. Thaw the vial containing Benzonase® Nuclease solution and add 10 µl to the 10 ml aliquot of native Lysis Buffer.

Materials and equipment to be supplied by user

- *E. coli* cell pellet from 250 ml culture
- 2x SDS-PAGE sample buffer

Procedure

1. **Thaw the cell pellet for 15 min on ice and resuspend the cells in 10 ml native Lysis Buffer.**

   Ensure that lysozyme and Benzonase® Nuclease have been added to the lysis buffer.

2. **Incubate on ice for 30 min. Mix 2–3 times by gently swirling the cell suspension.**
3. **Centrifuge lysate at 14,000 x *g* for 30 min at 4°C to pellet the cellular debris. Retain the cell lysate supernatant.**

   The supernatant contains the soluble fraction of the recombinant protein.

4. **Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of the supernatant and store at –20°C for SDS-PAGE analysis.**
5. **Gently resuspend the resin in a Fast Start Column by inverting it several times.**
6. **Break the seal at the outlet of the column, open the screw cap, and allow the storage buffer to drain out.**

   It is important that the outlet seal is broken before the screw cap is removed.

7. **Apply the cell lysate supernatant from step 3 to the column.**
8. **Collect the flow-through fraction. Add 5 µl 2 x SDS-PAGE sample buffer to a 5 µl aliquot of the flow-through fraction and store at –20°C for SDS-PAGE analysis.**

9. **Wash the column 2 times with 4 ml of native Wash Buffer. Collect both wash fractions. Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of each wash fraction and store at –20°C for SDS-PAGE analysis.**
10. **Elute bound 6xHis-tagged protein with two 1 ml aliquots of Native Elution Buffer.**
11. **Collect each elution fraction in a separate tube. Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of each elution fraction and store at –20°C for SDS-PAGE analysis.**
12. **Analyze all fractions by SDS-PAGE.**

Protocol: Purification of 6xHis-tagged Proteins Under Denaturing Conditions

Things to do before starting

Before use, equilibrate Denaturing Buffer to ambient temperature, and dissolve any precipitate by gentle stirring. Directly before commencing the purification protocol, lysis, wash, and elution buffers must be prepared as follows:

Wash buffer: Transfer 60 ml of Denaturing Buffer to a clean bottle and adjust the pH to 6.3 using concentrated HCl. Label the bottle "Denaturing Wash Buffer, pH 6.3".

Elution buffer: Transfer 30 ml of Denaturing Buffer to a clean bottle and adjust the pH to 4.5 using concentrated HCl. Label the bottle "Denaturing Elution Buffer, pH 4.5".

Lysis buffer: Adjust the pH of the remaining Denaturing Buffer to 8.0 using concentrated NaOH. Label the bottle "Denaturing Lysis Buffer, pH 8.0".

Once prepared, Denaturing Lysis, Wash, and Elution Buffers should be stored at 2–8°C to prevent pH changes due to hydrolysis of urea. The pH of each buffer should be checked (and where necessary adjusted) before each purification procedure.

Materials and equipment to be supplied by user

- *E. coli* cell pellet from 250 ml culture
- 2x SDS-PAGE sample bufferProcedure

Procedure

1. **Resuspend the cell pellet in 10 ml Denaturing Lysis Buffer.**
2. **Incubate at room temperature (15–25°C) for 60 min. Mix 2–3 times by gently swirling the cell suspension.**
   Lysis is complete when the suspension is translucent.
3. **Centrifuge lysate at 14,000 x *g* for 30 min at room temperature (15–25°C) to pellet the cellular debris. Retain the cell lysate supernatant.**
   The supernatant contains the recombinant protein.
4. **Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of the supernatant and store at –20°C for SDS-PAGE analysis.**
5. **Gently resuspend the resin in a Fast Start Column by inverting it several times.**

6. **Break the seal at the outlet of the column, open the screw cap, and allow the storage buffer to drain out.**

   It is important that the outlet seal is broken before the screw cap is removed.

7. **Apply the cell lysate supernatant from step 3 to the column.**
8. **Collect the flow-through fraction. Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of the flow-through fraction and store at –20°C for SDS-PAGE analysis.**
9. **Wash the column 2 times with 4 ml of Denaturing Wash Buffer. Collect both wash fractions. Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of each wash fraction and store at –20°C for SDS-PAGE analysis.**
10. **Elute bound 6xHis-tagged protein with two 1 ml aliquots of Denaturing Elution Buffer.**
11. **Collect each elution fraction in a separate tube. Add 5 µl 2x SDS-PAGE sample buffer to a 5 µl aliquot of each elution fraction and store at –20°C for SDS-PAGE analysis.**
12. **Analyze all fractions by SDS-PAGE.**

Analyzing Recombinant Protein Expression and Purification

Expression analysis is most easily performed using SDS polyacrylamide gel electrophoresis (SDS-PAGE). Using this technique, proteins are separated according to their size in a polyacrylamide gel matrix. After separation, proteins can be visualized using a universal staining technique, such as silver or Coomassie® staining (see protocol, page 24).

If proteins are poorly resolved or present in low amounts, individual proteins can be visualized using antibodies, in a technique called western blotting. This technique involves transfer of proteins from the gel to a nitrocellulose membrane and probing with antibodies specific to a protein or affinity-tag epitope. The Ni-NTA Fast Start Kit contains the Penta·His Antibody which recognizes an epitope of five consecutive histidine residues. This antibody can be used for immunodetection of 6xHis-tagged protein expressed from any pQE vector.

6xHis Protein Ladder

When analyzing the expression of 6xHis-tagged proteins, the 6xHis Protein Ladder (cat. no. 34705) serves as a molecular weight standard and a positive control for western blotting. The 6xHis Protein Ladder consists of five 6xHis-tagged proteins ranging from 15 to 100 kDa in size.

Protocol: Separation of Proteins by SDS-PAGE

The protocol below gives directions for mixing, pouring, and running an SDS-PAGE minigel. Several companies supply ready-to-use pre-cast gels. If using such a gel, ensure it is compatible with your electrophoresis equipment.

Materials and equipment to be supplied by user

- Gel apparatus and electrophoresis equipment
- 30% acrylamide/0.8% bis-acrylamide stock solution* — This can also be conveniently purchased as a ready-to-use solution from several companies (e.g., Rotiphorese Gel 30, Roth, cat. no. 3029.1)
- 2.5x separating gel buffer
- 5x stacking gel buffer
- TEMED (*N,N,N',N'*-tetramethylethylenediamine)
- 10% (w/v) ammonium persulfate
- Butanol
- 1x electrophoresis buffer
- Protein samples in SDS-PAGE sample buffer
- Heating block at 95°C

Note: Use only high-quality reagents and water for SDS-PAGE.

For buffer and reagent compositions, see Appendix, page 34.

Procedure

1. **Assemble gel plates with spacers according to the manufacturer's instructions.**
2. **Mark the level to which the separating gel should be poured — a few millimeters below the level where the wells will be formed by the comb.**

   The size of the gel apparatus used will determine the volumes of gel solutions necessary. The following are used for a 12% acrylamide 8 x 8 or 8 x 10 cm, 1mm thick, minigel.

* Acrylamide is a potent neurotoxin and is absorbed through the skin. Take appropriate safety measures particularly when weighing solid acrylamide/bisacrylamide, but also when working with the solutions and gels.

3. **For a 12% acrylamide gel, mix the following in a beaker or similar vessel.**

   2.2 ml 30% acrylamide/0.8% bis-acrylamide stock solution

   2.2 ml 2.5x separating gel buffer

   1.1 ml distilled water

   5 µl TEMED

4. **Add 50 µl 10% ammonium persulfate, and mix well, just before pouring between the assembled gel plates to the level marked in step 2. Overlay with butanol.**

   As soon as ammonium persulfate is added the gel should be poured quickly before the acrylamide polymerizes. Water can be used instead of butanol when using apparatus that may be damaged by the use of butanol — see the manufacturer's instructions.

5. **After polymerization is complete, pour off butanol, rinse with water and dry.**

   Water remaining on the plates can be removed using pieces of filter paper.

6. **For the stacking gel, mix the following:**

   0.28 ml 30% acrylamide stock solution

   0.33 ml 5x stacking gel buffer

   1 ml distilled water

   2 µl TEMED

7. **Add 15 µl 10% ammonium persulfate, and mix well, just before pouring on top of separating gel. Insert comb, avoiding introduction of air bubbles.**

   As soon as ammonium persulfate is added the stacking gel should be poured quickly before the acrylamide polymerizes.

8. **After the stacking gel polymerizes, the gel can be placed in the electrophoresis chamber. Fill the chamber with 1x electrophoresis buffer, and remove comb.**

9. **Heat each protein sample at 95°C for 5 min.**

   Ensure that SDS-PAGE sample buffer has been added to protein samples.

10. **Load samples and run the gel until the dye front reaches the bottom of the gel. For electrophoresis conditions refer to the recommendations provided by the manufacturer of the apparatus.**

11. **Visualize protein either by Coomassie staining (see protocol on page 24) or by immunodetection using Penta·His Antibody following western blotting (see protocols on pages 25–30).**

Protocol: Coomassie Staining of SDS-PAGE Gels

Note: Once stained with Coomassie staining solution, proteins cannot be transferred to a membrane by western blotting and detected using Anti·His antibodies.

Materials to be supplied by user

- Coomassie staining solution
- Destaining solution
- SDS polyacrylamide gel containing separated proteins

For buffer and reagent compositions, see Appendix, page 34.

Procedure

1. **Carefully disassemble the glass plates and spacers and remove the gel. Incubate the gel in Coomassie staining solution for between 30 min and 2 h with gentle shaking.**

   Coomassie Brilliant Blue R reacts nonspecifically with proteins.

2. **Remove the gel from the Coomassie staining solution and gently agitate the stained gel in destaining solution until the background becomes clear (1–2 h).**

   A folded paper towel placed in the destaining bath will soak up excess stain and allow the re-use of destaining solution.

   After destaining the proteins appear as blue bands against a clear gel background. Typically, bands containing 50 ng protein can be visualized.

SDS-PAGE Separation and Coomassie Staining of Proteins

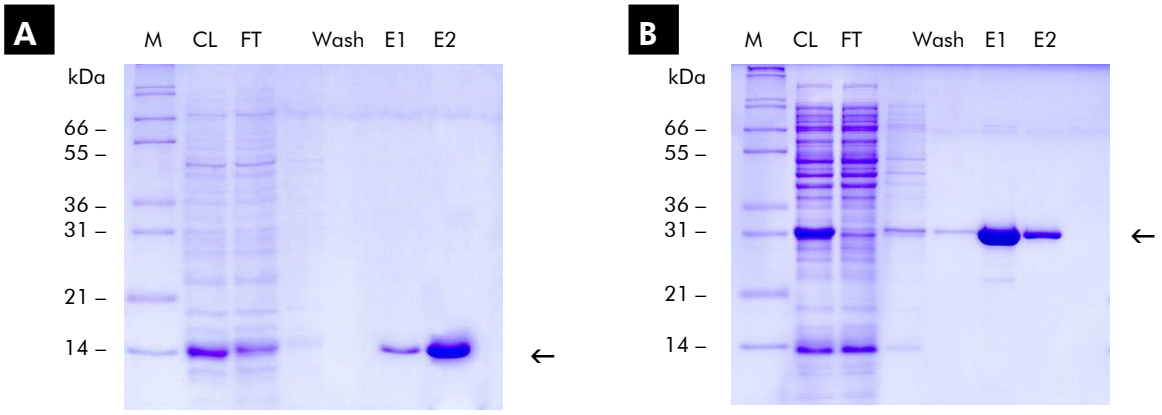


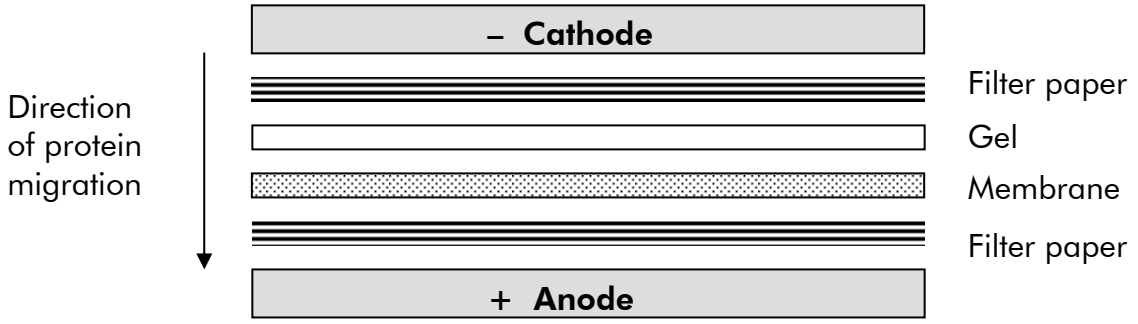
Figure 2 Coomassie-stained SDS-PAGE gels showing **A** 6xHis-tagged thioredoxin purified under denaturing conditions, and **B** 6xHis-tagged green fluorescent protein (GFP) purified under native conditions. **M**: markers, **CL**: cell lysate, **FT**: flow-through fraction, **Wash**: wash fractions, **E1** and **E2**: elution fractions. 5 µl of each fraction was loaded on to the gel.

Protocol: Western Transfer

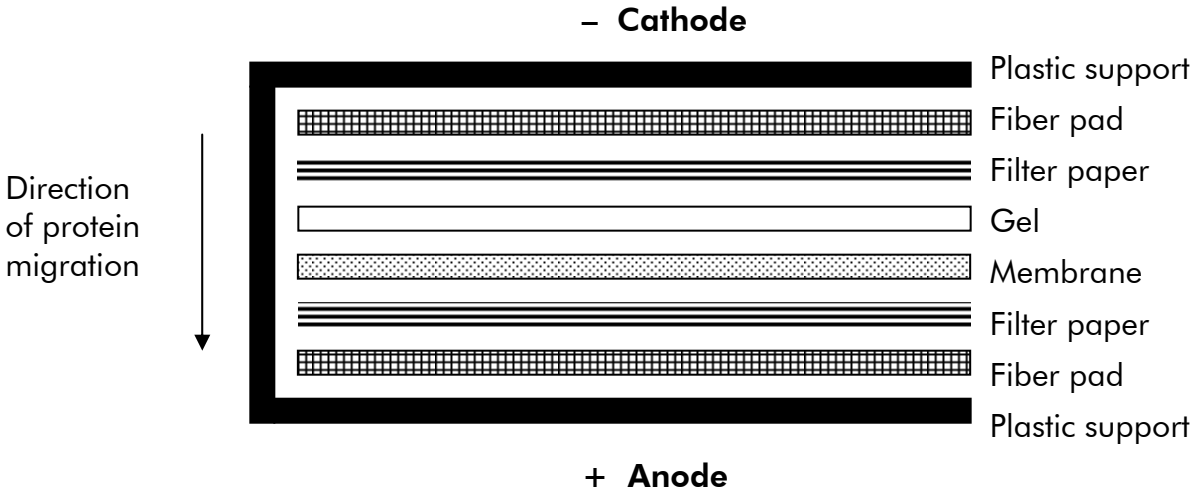
Principle

Following electrophoresis, proteins in a polyacrylamide gel can be transferred to a positively charged nitrocellulose membrane (e.g., Schleicher and Schuell BA85) by semi-dry electroblotting or in a buffer-tank–blotting apparatus.

With the semi-dry electroblotting method, the gel and membrane are sandwiched between two stacks of filter paper that have been pre-wet with transfer buffer (see below). The membrane is placed near the anode (positively charged), and the gel is placed near the cathode (negatively charged). SDS-coated, negatively charged proteins are transferred to the membrane when an electric current is applied.



With the tank blotting method, a blotting cassette is submerged in a tank for blotting (see below). Tank blotting can be performed over extended periods since the buffer capacity is far greater than that with semi-dry transfer systems. Results obtained with the tank blotting method are typically better, with more efficient transfer, particularly of large proteins. Transfer efficiency can be checked by staining proteins on the membrane using Ponceau S (see protocol, page 28). Once transferred to the membrane, the proteins can be probed with epitope-specific antibodies or conjugates.



Materials and equipment to be supplied by user

- Transfer apparatus
- Filter paper
- Nitrocellulose membrane (e.g., Schleicher and Schuell BA85)
- Polyacrylamide gel containing separated proteins
- Transfer buffer (semi-dry or tank-blotting)

For buffer and reagent compositions, see Appendix, page 34.

Protocols are given for semi-dry transfer and tank-blotting procedures.

Semi-dry–transfer procedure

1. **Cut 8 pieces of filter paper and a piece of membrane to the same size as the gel.**

   To avoid contamination, always handle the filter paper, gel, and membrane with gloves.

2. **Incubate membrane for 10 min in semi-dry–transfer buffer.**
3. **Soak filter paper in semi-dry–transfer buffer.**
4. **Avoiding air bubbles, place 4 sheets of filter paper on the cathode (negative, usually black), followed by the gel, the membrane, 4 sheets of filter paper, and finally the anode (positive, usually red). Air bubbles may cause localized nontransfer of proteins. They can be removed by gently rolling a Pasteur pipette over each layer in the sandwich.**
5. **For current, voltage, and transfer times consult the manufacturer's instructions.**

   Time of transfer is dependent on the size of the proteins, percentage acrylamide, and gel thickness. Transfer efficiency should be monitored by Ponceau S staining (see page 28). The field strength required is determined by the surface area and thickness of the gel: 0.8 mA/cm^2 is a useful guide (1 h transfer).

6. **After transfer, mark the orientation of the membrane on the gel.**

Tank-blotting procedure

1. **Cut 8 pieces of filter paper and a piece of membrane to the same size as the gel.**

   To avoid contamination, always handle the filter paper, gel, and membrane with gloves.

2. **Incubate membrane for 10 min in tank-blotting transfer buffer.**
3. **Soak filter paper and membrane in tank-blotting transfer buffer.**
4. **Avoiding air bubbles, place 4 sheets of filter paper on the fiber pad, followed by the gel, the membrane, 4 sheets of filter paper, and finally the other fiber pad.**

   Air bubbles may cause localized nontransfer of proteins. They can be removed by gently rolling a Pasteur pipette over each layer in the sandwich.

5. **For current, voltage, and transfer times consult the manufacturer's instructions.**

   Time of transfer is dependent on the size of the proteins, percentage acrylamide, and gel thickness. Transfer efficiency should be monitored by Ponceau S staining (see protocol, page 28). The field strength required is determined by the surface area and thickness of the gel: 0.8 mA/cm^2 is a useful guide (1 h transfer).

6. **After transfer, mark the orientation of the gel on the membrane.**

Protocol: Staining Proteins after Western Transfer

Materials and equipment to be supplied by user

- Staining solution: 0.5% (w/v) Ponceau S, 1% (v/v) glacial acetic acid
- Western blot

Procedure

1. **Incubate membrane in staining solution (0.5% [w/v] Ponceau S, 1% [v/v] glacial acetic acid) with gentle agitation for 2 min.**
2. **Destain in distilled water until bands are visible.**

   If the 6xHis Protein Ladder has been used as a positive control, the bands should be weakly visible after this staining procedure. Check that the proteins of different sizes have been transferred uniformly to the membrane.
3. **Mark membrane or cut as desired.**

   The orientation of the membrane on the gel should be marked. It is not necessary to mark the positions of bands of the 6xHis Protein Ladder. Their positions will be clear after the 6xHis-tagged protein detection procedure.
4. **Proceed with the protocol on page 29.**

   The blot will be destained in the washing or blocking solution at the beginning of the immunological detection protocol. If the membrane is to be stored at this stage it should be blocked and washed (step 1–4), dried, and then stored at 4°C. The length of time that the blot can be stored is dependent on the samples on the blot.

Protocol: Immunodetection of 6xHis-tagged Proteins with Penta·His Antibody (Chemiluminescent Method)

Of the two most commonly used immunodetection methods (chemiluminescent and chromogenic detection), chemiluminescence is the more sensitive. A protocol for chromogenic detection of 6xHis-tagged proteins and a comprehensive Troubleshooting Guide can be found in the *QIAexpress Detection and Assay Handbook*.

Materials and equipment to be supplied by user*

- Western blot
- TBS Buffer
- TBS-Tween/Triton Buffer
- Blocking buffer
- Anti-mouse secondary antibody conjugate. Either alkaline phosphatase (AP) or horseradish peroxidase (HRP) conjugated anti-mouse IgG may be used. Rabbit-anti-mouse IgG/AP-conjugate from Pierce (Cat. No. 31332) or goat-anti-mouse IgG/HRP-conjugate from Jackson Immunoresearch (Cat. No. 115-035-003) yield good results.
- Secondary antibody dilution buffer

For chemiluminescent detection, BSA does not sufficiently block nonspecific binding of the secondary antibody to the membrane, and milk powder should be used to dilute the secondary antibody. Buffer containing milk powder should not be used for Penta·His Antibody dilution as this will reduce sensitivity. If alkali-soluble casein (Merck, Cat. No. 1.02241) is available in your country it can be used as a blocking reagent throughout the entire chemiluminescent detection protocol.

Chemiluminescent substrates

Please refer to manufacturer's recommendations. CDP-Star™ (e.g., from Applied Biosystems) can be used with AP-conjugated secondary antibodies, and the ECL™ system from Amersham Biosciences can be used in combination with HRP-conjugated secondary antibodies. The blocking reagents supplied with the CDP-Star system are compatible with Penta·His Antibodies and can be used, according to the manufacturer's instructions, instead of the blocking buffers and secondary antibody dilution buffers described in the following protocol.

Table 3. Reagents Used for Chemiluminescent Detection

Step	Reagent required
Blocking	3% BSA in TBS or 1% casein in TBS
Penta·His Antibody binding	3% BSA in TBS or 1% casein in TBS
Secondary antibody binding	10% milk powder in TBS or 1% casein in TBS

Procedure

1. **Wash membrane twice for 10 min each time with TBS buffer at room temperature (15–25°C).**
2. **Incubate for 1 h in blocking buffer at room temperature.**
   3% BSA (w/v) in TBS buffer*, is used for blocking until incubation.
3. **Wash membrane twice for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**
4. **Wash membrane for 10 min with TBS buffer at room temperature.**
5. **Incubate membrane in Penta·His Antibody solution (1/1000–1/2000 dilution of antibody or conjugate stock solution in blocking buffer) at room temperature (15–25°C) for 1 h.**
   Membrane can be sealed in plastic bags. **Note**: Do not use buffer containing milk powder for Penta·His Antibody dilution. This will reduce sensitivity. 3% BSA (w/v) in TBS buffer* is used for this blocking step when using chemiluminescent detection.
6. **Wash twice for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**
7. **Wash for 10 min in TBS buffer at room temperature(15–25°C).**
8. **Incubate with secondary antibody solution for 1 h at room temperature (15–25°C).**
   Dilute according to the manufacturer's recommendations. Use the lowest recommended concentration to avoid false signals.

   10% nonfat dried milk in TBS* is used for incubation with secondary antibody when using chemiluminescent detection. Milk powder is needed to reduce background because BSA does not block sufficiently for the very sensitive chemiluminescent detection method.
9. **Wash 4 times for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**
10. **Perform chemiluminescent detection reaction and expose to X-ray film according to the manufacturer's recommendations.**

* If alkali -soluble casein (Merck, Cat. No. 1.02241) is available in your country a 1% (w/v) solution in TBS buffer may be used for this protocol step.

Troubleshooting Guide — Expression and Purification

The following troubleshooting guide may be helpful in solving any problems that may arise. The scientists at QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications in general (see back cover for contact details).

	Comments and suggestions
No or low expression	
a) Protein is poorly expressed	Check that the protein is not found in the insoluble fraction.
b) Culture conditions for expression are incorrect	Use the same culture conditions and host cells to check the expression of DHFR encoded by a control plasmid (pQE-40).
c) Coding sequence is ligated into the incorrect reading frame	Sequence the ligated junctions.
d) Protein is secreted	Remove all signal sequences from the coding region.
e) Protein is rapidly degraded	Perform a time course to check the kinetics of growth and induction. If the protein is small (<10 kDa), consider adding an N-terminal carrier protein such as DHFR. If degradation occurs after cell lysis, consider adding protease inhibitors. Keep all samples and solutions at 4°C.
Inclusion bodies are formed	
a) Protein is highly toxic	Use *E. coli* M15 [pREP4] in combination with one of the pQE-80L series of expression vectors.

	Comments and suggestions
b) Expression level is too high (protein cannot fold correctly)	Reduce expression levels by modifying growth and induction conditions. Protein is insoluble. Check both soluble and insoluble fractions for protein. Try to solubilize protein with denaturants or detergents. **Note**: It may not be necessary to use denaturing conditions for purification if the protein of interest is insoluble or has formed inclusion bodies. Check the levels of soluble protein remaining in the cytoplasm.
Protein does not bind to the Ni-NTA Fast Start 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 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.

	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	
a) Protein has precipitated on the column	Elute under denaturing conditions.
b) Protein is still bound to the column	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: 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 preparing competent *E. coli*

TFB1	100 mM RbCl; 50 mM $MnCl_2$; 30 mM potassium acetate; 10 mM $CaCl_2$; 15% glycerol, adjust to pH 5.8*, sterile-filter
TFB2	10 mM MOPS; 10 mM RbCl; 75 mM $CaCl_2$; 15% glycerol, adjust to pH 6.8 with KOH, sterile filter

SDS-PAGE sample buffers

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
5x SDS-PAGE sample buffer	0.225 M Tris·Cl pH 6.8; 50% glycerol; 5% SDS; 0.05% bromophenol blue; 0.25 M DTT

* Adjust pH carefully as insoluble Mn precipitates can form.

Solutions for SDS-PAGE

30% acrylamide/ 0.8% bis-acrylamide stock solution	30% acrylamide/0.8% bis-acrylamide (N,N'-methylene-bis-acrylamide) (e.g., Roth, Cat. No. 3029.1)
2.5x separating gel buffer	1.875 M Tris·Cl pH 8.9; 0.25% (w/v) SDS
5x stacking gel buffer	0.3 M Tris-phosphate pH 6.7; 0.5% (w/v) SDS
5x electrophoresis buffer	0.5 M Tris base; 1.92 M glycine; 0.5% (w/v) SDS should be pH 8.8. Do not adjust.

Solutions for western transfer

Semi-dry transfer buffer	25 mM Tris base; 150 mM glycine; 10% methanol. Should be pH 8.3 without adjusting.
Tank-blotting transfer buffer	25 mM Tris base; 150 mM glycine; 20% methanol. Should be pH 8.3 without adjusting.

Solutions for detection procedures

TBS buffer	10 mM Tris·Cl, pH 7.5; 150 mM NaCl
TBS-Tween buffer	20 mM Tris·Cl, pH 7.5; 500 mM NaCl; 0.05% (v/v) Tween 20 (Sigma, Cat. No. P1379)
TBS-Tween/Triton buffer	20 mM Tris·Cl, pH 7.5; 500 mM NaCl; 0.05% (v/v) Tween 20; 0.2% (v/v) Triton X-100 (Sigma, Cat. No. X-100)
Blocking buffers	3% (w/v) BSA (Sigma, Cat. No. A7906) in TBS buffer 1% (w/v) alkali-soluble casein (Merck, cat. no. 1.02241) in TBS Alkali-soluble casein is not easily dissolved in TBS. Dissolve casein and NaCl in 10 mM Tris base, and then adjust pH if necessary.
Secondary antibody dilution buffer for chemiluminescent detection	10% (w/v) skim milk powder (Fluka, cat. no. 70166) in TBS buffer. For best results, milk powder should be dissolved overnight at 4°C.

Coomassie staining solutions

Coomassie staining solution	0.05% (w/v) Coomassie Brilliant Blue R-250*; 40% (v/v) ethanol; 10% (v/v) glacial acetic acid. For 1 liter, dissolve 500 mg Coomassie Brilliant Blue R-250 in 400 ml 100% ethanol. Add 100 ml glacial acetic acid and water to 1 liter. Filter before use.
Destaining solution	40% (v/v) ethanol; 10% (v/v) glacial acetic acid

* e.g., SIGMA, cat. no. B 0149

Ordering Information

Product	Contents	Cat. no.
Ni-NTA Fast Start Kit (6)	For purification and detection of six 6xHis-tagged protein preps: 6 x Fast Start Columns, 3 µg Penta·His Antibody, Buffers and Reagents	30600
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
***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
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
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

Product	Contents	Cat. no.
Penta·His HRP Conjugate Kit	125 µl Penta·His HRP Conjugate, 5 g Blocking Reagent, 50 ml Blocking Reagent Buffer, 10x Concentrate	34460
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.

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)

1069563 07/2011

Sample & Assay Technologies