

## ***Strep-tag* Protein Handbook**

For expressing, purifying, and detecting  
proteins carrying a *Strep-tag*<sup>®</sup> II

Two-step protein purification system

His·*Strep* pQE-TriSystem Vector Set

pQE-TriSystem *Strep* Vector

*Strep-Tactin*<sup>®</sup> Superflow Plus and *Strep-Tactin*

Superflow Plus Cartridges

*Strep-Tactin* Magnetic Beads

*Strep-tag* Antibody



## **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](http://www.qiagen.com).

# Contents

Kit Contents	5
Storage	6
Product Use Limitations	6
Quality Control	6
Product Warranty and Satisfaction Guarantee	7
Technical Assistance	7
Safety Information	8
Introduction	9
Protocols	
■ Growth of <i>E. coli</i> Cultures (50 ml)	13
■ Preparation of Cleared <i>E. coli</i> Lysates Under Native Conditions	14
■ Preparation of Cleared Lysates from Baculovirus-Infected Insect Cells	17
■ Preparation of Lysates from Transfected Mammalian Cells	19
■ Manual Purification of <i>Strep</i> -tagged Proteins Using a Syringe	21
■ Purification of <i>Strep</i> -tagged Proteins Using Liquid Chromatography Systems	23
■ Batch Purification of <i>Strep</i> -tagged Proteins Using <i>Strep</i> -Tactin Superflow Plus	25
■ Micro-Scale Purification of <i>Strep</i> -tagged Proteins Using <i>Strep</i> -Tactin Magnetic Beads	26
■ Immunodetection Using the <i>Strep</i> -tag Antibody (Chemiluminescent Method)	28
■ Immunodetection Using the <i>Strep</i> -tag Antibody (Chromogenic Method)	31
Troubleshooting Guide	33
Appendix A: Two-Step Purification Using a His-tag and a <i>Strep</i> -tag	39
■ Protein Purification from Insect-Cell Growth Medium	43
■ Manual Purification of His-tagged Proteins Using a Syringe	44
■ Purification of His-tagged Proteins Using Liquid Chromatography Systems	46
■ Batch Purification of His- <i>Strep</i> -tagged Proteins Using Ni-NTA Matrices	48

■ <b>Micro-Scale Purification of His-<i>Strep</i>-tagged Proteins Using Ni-NTA Magnetic Agarose Beads</b>	<b>50</b>
<b>Appendix B: Buffer Compositions</b>	<b>52</b>
<b>Appendix C: Regeneration of <i>Strep</i>-Tactin Superflow Plus</b>	<b>57</b>
<b>Ordering Information</b>	<b>58</b>

## Kit Contents

<b><i>Strep-Tactin Superflow Plus Cartridge</i></b>	<b>(1 ml)</b>	<b>(5 ml)</b>
<b>Catalog no.</b>	<b>30020</b>	<b>30060</b>
<i>Strep-Tactin Superflow Plus Cartridge</i>	1 x 1 ml	1 x 5 ml
Handbook	1	1

<b><i>Strep-Tactin Superflow Plus</i></b>	<b>(2 ml)</b>	<b>(10 ml)</b>
<b>Catalog no.</b>	<b>30002</b>	<b>30004</b>
<i>Strep-Tactin Superflow Plus</i>	2 ml	10 ml
Handbook	1	1

<b><i>Strep-Tactin Magnetic Beads</i></b>	
<b>Catalog no.</b>	<b>36311</b>
<i>Strep-Tactin Magnetic Beads</i>	2 x 1 ml
Handbook	1

<b><i>Strep-tag Antibody</i></b>	
<b>Catalog no.</b>	<b>34850</b>
Mouse monoclonal antibody that recognizes the <i>Strep-tag II</i> epitope; lyophilized, for 1000 ml working solution	100 $\mu$ g
Product sheet	1
Handbook	1

## Storage

*Strep-Tactin* matrices should be stored at 2–8°C. Under these conditions, *Strep-Tactin* matrices can be stored for up to 6 months without any reduction in performance. *Strep-Tactin* matrices should not be frozen.

*Strep-tag* Antibodies should be stored lyophilized until they are ready to be used. They can be stored lyophilized for 6 months at 2–8°C. In solution, they can be stored for 3 months at 2–8°C or for 6 months in aliquots at –20°C. Avoid repeated freezing and thawing.

pQE-TriSystem His-*Strep* Vectors are supplied lyophilized with sucrose and bromophenol blue for visualization, and should be resuspended in a convenient volume of TE buffer (e.g., 10 µl) and stored at –20°C. Sucrose and bromophenol blue do not interfere with restriction digestions or bacterial transformation.

## Product Use Limitations

The kits and reagents described in this handbook are intended for molecular biology applications. These products are 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.

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of kit and reagents described in this handbook is tested against predetermined specifications to ensure consistent product quality.

## Product Warranty and Satisfaction Guarantee

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

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

## Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the products described in this handbook or QIAGEN products in general, please do not hesitate to contact us.

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

For technical assistance and more information, please see our Technical Support Center at [www.qiagen.com/Support](http://www.qiagen.com/Support) or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

## 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/Support/MSDS.aspx](http://www.qiagen.com/Support/MSDS.aspx) where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

The following risk and safety phrases apply to *Strep-tag* Antibodies.

### **Strep-tag Antibodies**

Sensitizer. Risk and safety phrases:\* R42/43. S24-26-36/37

### **24-hour emergency information**

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

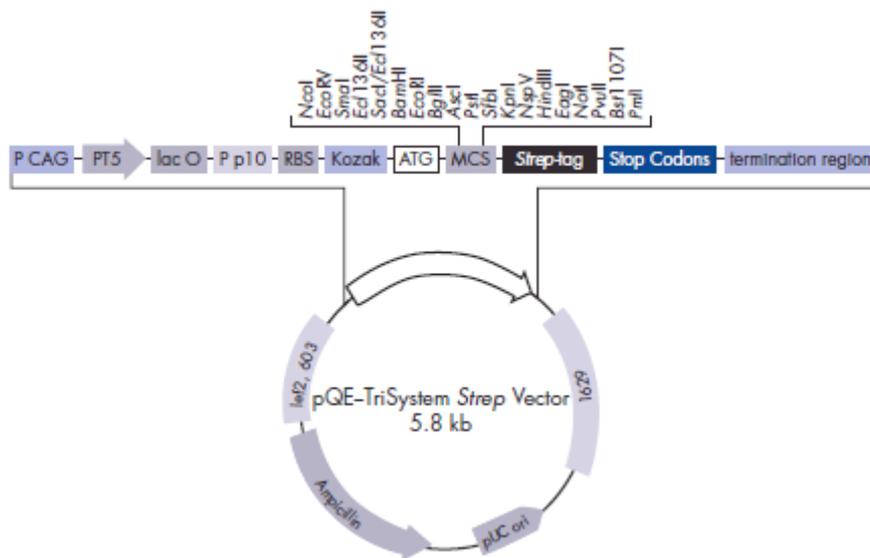
Poison Information Center Mainz, Germany

Tel: +49-6131-19240

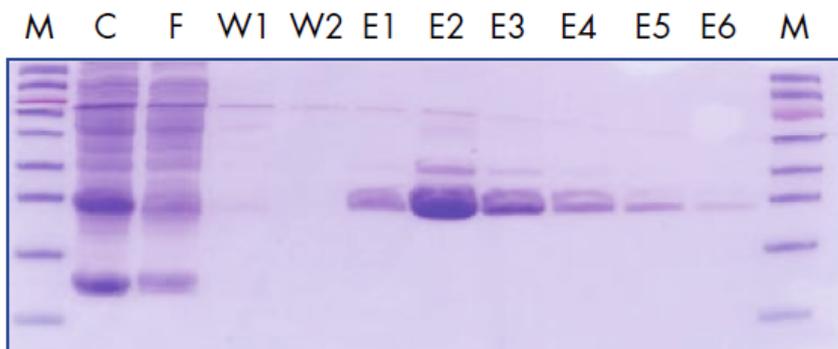
\* R42/43: May cause sensitization by inhalation and skin contact. S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37: Wear suitable protective clothing and gloves.

## Introduction

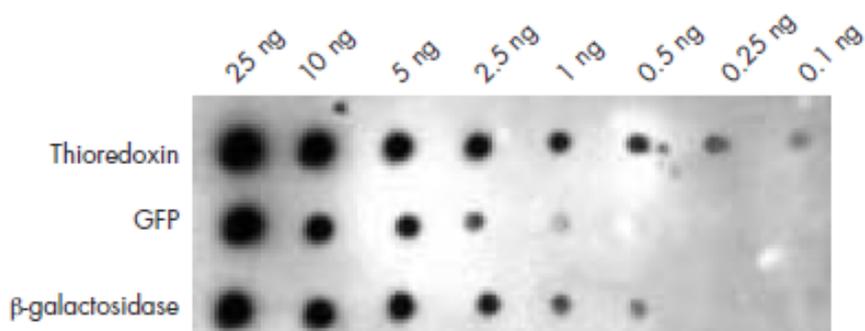
The *Strep*-tag II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to *Strep*-Tactin, an engineered streptavidin. The *Strep*-tag II allows affinity chromatography on immobilized *Strep*-Tactin under physiological conditions, enabling native, active *Strep*-tagged proteins can be purified in a single step. The binding affinity of the *Strep*-tag II to *Strep*-Tactin ( $K_d = 1 \mu\text{M}$ ) is nearly 100 times higher than to streptavidin. After a short washing step, gentle elution of purified recombinant protein is performed by addition of low concentrations of biotin or desthiobiotin. Desthiobiotin is a stable, reversibly binding analog of biotin, the natural ligand of streptavidin. Proteins containing the *Strep*-tag II epitope can be detected with high specificity and sensitivity using *Strep*-tag Antibodies (Figure 3, page 10).



**Figure 1. pQE-TriSystem *Strep* vector for parallel protein expression using a single construct in *E. coli*, insect, and mammalian cells.** **PT5:** T5 promoter; **lac O:** lac operator; **RBS:** Ribosome binding site; **ATG:** Start codon; **MCS:** Multiple cloning site, ***Strep*-tag:** *Strep*-tag sequence; **Stop Codons:** Stop codons in all three reading frames; **Ampicillin:** Ampicillin resistance gene; **P CAG:** CMV/actin/globin promoter; **P p10:** p10 promoter; **Kozak:** Kozak consensus sequence; **Termination region:** Transcription terminator region; **lef2, 603/1629:** Flanking baculovirus sequences to permit generation of recombinant baculoviruses; **pUC ori:** pUC origin of replication.



**Figure 2. Efficient purification of Strep-tagged GFP with Strep-Tactin Superflow Plus.** GFP was expressed in 60 ml *E. coli* culture and a 5 ml aliquot was applied to Strep-Tactin Superflow Plus. Protein was eluted using 25 mM desthiobiotin, giving a total protein yield of 3.1 mg. **C:** Cleared lysate, **E:** Elution fractions; **F:** Flow-through; **M:** Markers; **W:** Wash.



**Figure 3. Dot blot showing the sensitivity of Strep-tag Antibody.** The indicated amounts of protein were spotted onto a membrane and detected using Strep-tag Antibody, an anti-mouse secondary antibody conjugated to horseradish peroxidase (HRP), and the ECL™ chemiluminescent detection system.

## Purification scale

Protocols are provided in this handbook for lysis of *E. coli*, insect, and mammalian cells. The purification scale is dependent on the amount of protein in the preparation. A column size and total binding capacity should be chosen to approximately match the amount of protein to be purified (see Table 1). Very few nontagged proteins will be retained on the resin when nearly all available binding sites are occupied by the tagged protein. If too much matrix is used, other proteins may bind nonspecifically to unoccupied sites and elute as contaminants.

High-yield preparations from *E. coli* cultures can be purified using a batch or fast protein liquid chromatography (FPLC) procedure. Eukaryotic expression systems typically deliver smaller amounts of protein than prokaryotic systems. This requires that smaller amounts of purification matrix be used for high-efficiency purification. Proteins that are obtained in high yields from baculovirus-infected insect cells can be purified using batch procedures. However, if protein expression is low, a purification procedure using magnetic beads is the method of choice. *Strep*-tagged proteins expressed in mammalian cells should be purified using *Strep*-Tactin Magnetic Beads. Table 1 lists the protein binding capacities of *Strep*-Tactin matrices. The new *Strep*-Tactin Superflow Plus has a 3-fold increased binding capacity.

**Table 1. Protein binding capacities of *Strep*-Tactin matrices**

<b>Matrix</b>	<b>Protein binding capacity</b>
<i>Strep</i> -Tactin Superflow Plus	Up to 9 mg/ml
<i>Strep</i> -Tactin Superflow	Up to 3 mg/ml (~150 nmol @ 20 kDa)
<i>Strep</i> -Tactin Magnetic Beads	200–300 $\mu$ g/ml (10% suspension) (~10–15 nmol @ 20 kDa)

## Buffer and reagent compatibility

Some reagents and buffer components used for protein purification may interfere with the affinity interaction between the protein and purification matrix. Table 2 lists compatibility of various reagents with the *Strep*-tag–*Strep*-Tactin interaction.

**Table 2. Reagents compatible with the *Strep*-tag/*Strep*-Tactin interaction**

<b>Nonionic detergents</b>	<b>Ionic and zwitterionic detergents</b>	<b>Reducing agents</b>	<b>Others</b>	<b>Salts</b>
2% Triton X-100	2% N-lauryl-sarcosine	50 mM DTT	25% Glycerol	5 M Sodium chloride
2% Tween 20	0.1% SDS			2 M Ammonium sulfate
2% N-octyl- $\beta$ -D-glucopyranoside	1.3% N-octyl-2-hydroxyethylsulfoxide			1 M Calcium chloride
0.2% N-nonyl- $\beta$ -D-glucopyranoside	0.3% CHAPS			
2% Igepal <sup>®</sup> CA-630 (Nonidet P40)	0.1% N-dodecyl- N,N-dimethylamine-N-oxide	1 M Guanidine		
0.12% C10E5; Decylpentaoxyethylene	0.034% DDAO; N-decyl- N,N-dimethylamine-N-oxide	1 mM phenylmethylsulfonyl fluoride (PMSF)		
0.03% C10 E6		10% Ethanol		
0.005% C12 E8				
0.023% C12E9; Dodecyl nonaoxyethylene (Thesit)				
0.35% DM; Decyl- $\beta$ -D-maltoside				
0.007% LM; N-dodecyl $\beta$ -D-maltoside				

\* The reagents listed have been used successfully up to the concentrations given.

# Protocol: Growth of *E. coli* Cultures (50 ml)

## Important points before starting

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

## Equipment and reagents to be supplied by user

- LB medium
- Antibiotics
- Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG)

Buffer compositions are provided in Appendix B, page 52.

## Procedure

- 1. Inoculate 10 ml of LB medium containing the appropriate antibiotics in a 50 ml flask. Grow the cultures at 37°C overnight.**
- 2. Inoculate 50 ml of prewarmed media (with appropriate antibiotics) with 2.5 ml of the overnight cultures and grow at 37°C with vigorous shaking until an OD<sub>600</sub> 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  $\mu$ l 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  $\mu$ l 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.**

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

This lysis protocol uses *Strep-Tactin Superflow Lysis Buffer* and is suitable for subsequent single-step purification using a *Strep-Tactin* matrix.

### Important points before starting

- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.
- For ultrahigh-purity preparations we recommend a two-step purification procedure using *Ni-NTA Superflow* followed by further purification using *Strep-Tactin Superflow Plus* (see Appendix A, page 39). In such cases, use *Ni-NTA Superflow Lysis Buffer* for cell lysis.
- The amount of cells required depends on the expression level of the *Strep*-tagged protein. For proteins that are expressed at high levels (10–50 mg of tagged protein per liter of cell culture), a 10x concentrated cell lysate (resuspend the pellet from a 50 ml culture in 5 ml lysis buffer) can be used.
- Four ml of a 10x concentrated cell lysate in lysis buffer will contain approximately 0.4–2 mg of tagged protein.
- For much lower expression levels (1–5 mg/liter), 200 ml of cell culture should be used to obtain a 50x concentrated cell lysate (4 ml cell lysate = 0.2–1 mg of tagged protein).

### Equipment and reagents to be supplied by user

- 50 ml or 200 ml culture cell pellet (see above)
- *Strep-Tactin Superflow Lysis Buffer* (Buffer NP, see above)
- Lysozyme
- *Benzonase*<sup>®</sup> Nuclease (purity grade I, 25 U/ml, Merck, Germany, cat. no. 1.0169.0001)
- 2x SDS-PAGE sample buffer
- **Optional:** Sonicator

Buffer compositions are provided in Appendix B, page 52.

## Procedure

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

The amount of cells required depends on the expression level of the *Strep*-tagged protein and the expression system used. The binding capacity of *Strep*-Tactin resin is protein-dependent and normally lies around 3 mg/ml.

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

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

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

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

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

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

- 4. Add 5  $\mu$ l 2x SDS-PAGE sample buffer to 5  $\mu$ l supernatant and store at –20°C for SDS-PAGE analysis.**

- 5. Proceed using one of the purification protocols.**

See Table 3 to find the protocol best suited to your preparation scale.

**Table 3. Protocols for purification of His·Strep-tagged proteins from *E. coli* cell lysates**

	Expected amount of protein		
	$\leq 50 \mu\text{g}$	$> 50 \mu\text{g}$	$>> 50 \mu\text{g}$
Single-step purification	Magnetic bead protocol, page 26	Superflow Plus batch protocol, page 25	Cartridge protocols, page 23
Two-step purification:			
Ni-NTA	Magnetic bead protocol, page 50	Superflow Plus batch protocol, page 48	Cartridge protocols, page 46
<i>Strep</i> -Tactin	Magnetic bead protocol, page 26	Superflow Plus batch protocol, page 25	Cartridge protocols, page 23

## Protocol: Preparation of Cleared Lysates from Baculovirus-Infected Insect Cells

Since they provide posttranslational modifications, insect cells are often the eukaryotic expression system of choice. Although expression rates are normally significantly higher in insect cells than in mammalian cells, expressed-protein levels are typically lower than those obtained in bacterial systems. The estimated total protein content in insect cells is approximately 20 mg per  $10^7$  cells. With recombinant protein expression levels ranging between 0.05% and 50%, protein yields are typically 10  $\mu$ g – 10 mg per  $10^7$  cells.

**Note:** If the expected yield of the preparation is less than 50  $\mu$ g, it is highly recommended that proteins be purified using the protocols for *Strep-Tactin* Magnetic Beads (page 26). Larger amounts of protein can be purified using a batch procedure. Lysis Buffer supplemented with 1% Igepal CA-630 (Nonidet P40) is used for cell lysis. This lysis protocol uses *Strep-Tactin* Superflow Lysis Buffer and is suitable for subsequent single-step purification using a *Strep-Tactin* matrix.

For ultrahigh-purity preparations we recommend a two-step purification procedure using Ni-NTA Superflow followed by further purification using *Strep-Tactin* Superflow Plus (see Appendix A, page 39). In such cases, use Ni-NTA Superflow Lysis Buffer for cell lysis.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Cell pellet
- Phosphate-buffered saline (PBS)
- *Strep-Tactin* Superflow Lysis Buffer (Buffer NP) supplemented with 1% Igepal CA-630
- **Optional:** Benzonase Nuclease.

Buffer compositions are provided in Appendix B, page 52.

## Procedure

- 1. Wash the transfected cells with phosphate buffered saline (PBS) and collect them by centrifugation for 5 min at 1000 x g.**
- 2. Lyse the cells in lysis buffer supplemented with 1% Igepal CA-630 by incubating for 10 min on ice. Use 4 ml lysis buffer per 1–2 x 10<sup>7</sup> cells.**
- 3. (Recommended) If the lysate is very viscous, add Benzonase Nuclease (3 U per 1 x 10<sup>6</sup> cells) and incubate on ice for 10–15 min.**  
Alternatively, draw the lysate through a blunt-ended, narrow-gauge syringe needle several times.
- 4. Centrifuge the lysate at 10,000 x g for 10 min at 4°C to pellet cellular debris. Save the cleared lysate (supernatant).**  
The supernatant should contain the tagged protein.
- 5. Proceed using one of the purification protocols (see Table 3 to find the protocol best suited to your preparation scale).**

## Protocol: Preparation of Lysates from Transfected Mammalian Cells

Recombinant proteins are often expressed in mammalian cells to allow eukaryotic post-translational processing. However, expression levels are typically lower than in bacterial systems and often only small amounts of cell material are available. The total protein content in HeLa cells, for example, is around 3000  $\mu\text{g}$  per  $10^7$  cells. With recombinant protein expression levels at 0.01 to 1% of total protein, typical protein yields are 0.3 to 30  $\mu\text{g}$  per  $10^7$  cells. We therefore strongly recommend that proteins expressed in mammalian cells be purified using *Strep-Tactin* Magnetic Beads. Use of magnetic beads allows easy adjustment of binding capacity, and the small elution volumes provide concentrated proteins that are easily detected on SDS-PAGE gels.

This lysis protocol uses *Strep-Tactin* Beads Lysis Buffer (Buffer NP-T) and is suitable for subsequent single-step purification using *Strep-Tactin* Magnetic Beads.

For ultrahigh-purity preparations we recommend a two-step purification procedure using Ni-NTA Magnetic Agarose Beads followed by further purification using *Strep-Tactin* Magnetic Beads (see Appendix A). In such cases, use Ni-NTA Beads Lysis Buffer for cell lysis.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Cell pellet
- PBS
- *Strep-Tactin* Beads Lysis Buffer (Buffer NP)
- **Optional:** Benzonase Nuclease.

Buffer compositions are provided in Appendix B, page 52.

## Procedure

- 1. Wash the transfected cells with PBS and collect them by centrifugation for 5 min at 1000 x g.**
- 2. Resuspend the cells in lysis buffer (containing 0.05% Tween 20) using 500  $\mu$ l lysis buffer per  $10^7$  cells.**

If higher concentrations of nonionic detergent are required to solubilize the protein, detergents can be used in the lysis, wash, and elution buffers. Compatible detergents are Tween 20, Triton X-100, Igepal CA-630, and CHAPS (See Tables 2 and 6 on pages 12 and 42, respectively).

- 3. Lyse the cells according to steps 3a, 3b, or 3c.**

- 3a. Lyse the cells by sonication on ice.**

Use six 15 s bursts at 75 W with a 10 s cooling period between each burst. Use a sonicator equipped with a microtip.

- 3b. Lyse cells by three consecutive freeze–thaw cycles with freezing on dry ice and thawing at room temperature (15–25°C).**

- 3c. With 0.5–1% nonionic detergent (e.g., Tween 20 or Triton X-100) in the lysis buffer for solubilization, it is sufficient to incubate on an end-over-end shaker for 10 min at 4°C. Additional sonication or freeze–thaw cycles are not necessary.**

- 4. (Recommended) If the lysate is very viscous, add Benzonase Nuclease (3 U per  $1 \times 10^6$  cells) and incubate on ice for 10–15 min.**

Alternatively, draw the lysate through a blunt-ended, narrow-gauge syringe needle several times.

- 5. Centrifuge the lysate at 10,000 x g for 10 min at 4°C to pellet cellular debris. Save the supernatant.**

The supernatant should contain the *Strep*-tagged protein.

- 6. Proceed to protocol for purification using *Strep*-Tactin Magnetic Beads (page 26).**

## Protocol: Manual Purification of *Strep*-tagged Proteins Using a Syringe

This protocol is suitable for purifying *Strep*-tagged proteins using *Strep*-Tactin Superflow Plus Cartridges and a syringe. The composition of the lysis and elution buffers can be modified to suit the particular application, e.g., by adding Tween, DTT, or PMSF, or increasing NaCl or glycerol concentrations. See Table 2 on page 12 for a list of buffer reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction.

### Important points before starting

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

### Things to do before starting

- Immediately before applying to column, sterile filter (0.2 or 0.45  $\mu\text{m}$ ) or centrifuge the lysate (10,000  $\times g$ ) to ensure that it is particle-free.

### Equipment and reagents to be supplied by user

- Cleared cell lysate containing *Strep*-tagged protein
- *Strep*-Tactin Superflow Lysis Buffer (Buffer NP) and *Strep*-Tactin Superflow Elution Buffer (Buffer NPD). All buffers should be sterile filtered (0.2 or 0.45  $\mu\text{m}$ ) before use.
- 10 ml syringes
- Connector adapter

Buffer compositions are provided in Appendix B on page 52.

### Procedure

- 1. Fill syringe with Buffer NP, attach a suitable adapter, and expel air by depressing syringe plunger until buffer drips from the end of the adapter.**
- 2. Attach syringe to the cartridge inlet and remove cartridge outlet stopper.**
- 3. Equilibrate the cartridge with 10 column volumes of buffer.**

Do not use high pressure to force buffer through the cartridge. Ideal flow rates are 1 ml/min (1 ml cartridges) or 5 ml/min (5 ml cartridges).

- 4. Remove syringe and fill with cleared lysate. Apply cleared lysate to the cartridge using the same flow rate as in step 3.**

Collect and save the flow-through fraction.

- 5. Using a fresh syringe and the same flow rate as in steps 3 and 4, wash the cartridge with 10 column volumes of Buffer NP.**

Collect and save the wash fraction.

- 6. Fill a syringe with Buffer NPD and elute protein from cartridge using the recommended flow rate.**

Protein usually elutes within 5–10 column volumes. *Strep*-Tactin Superflow Plus Cartridges can be regenerated using the procedure provided in Appendix C on page 57.

# Protocol: Purification of *Strep*-tagged Proteins Using Liquid Chromatography Systems

This protocol is suitable for purifying *Strep*-tagged proteins using *Strep*-Tactin Superflow Plus Cartridges on liquid chromatography systems (such as the ÄKTA design™ or FPLC™ System). During equilibration, loading, washing, and elution, monitor the back pressure generated by the chromatography system. Do not allow back pressure to exceed 5 bar (0.5 MPa).

The composition of the lysis and elution buffers can be modified to suit the particular application, e.g., by adding Tween, DTT, or PMSF, or increasing NaCl or glycerol concentrations. See Table 2, page 12 for a list of buffer reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction.

## Important points before starting

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

## Equipment and reagents to be supplied by user

- Cleared cell lysate containing *Strep*-tagged protein. Immediately before applying it to the column, sterile filter (0.2 or 0.45  $\mu\text{m}$ ) or centrifuge the lysate (10,000  $\times g$ ) to ensure that it is particle-free.
- *Strep*-Tactin Superflow Lysis Buffer (Buffer NP) and *Strep*-Tactin Superflow Elution Buffer (Buffer NPD). All buffers should be sterile filtered (0.2 or 0.45  $\mu\text{m}$ ) before use.
- Connector adapter(s)

Buffer compositions are provided in Appendix B on page 52.

## Procedure

- 1. Fill system pumps with Buffer NP and attach cartridge to the pump outlet, taking care not to introduce air into the system.**
- 2. Remove cartridge outlet stopper and attach to the system tubing.**
- 3. Equilibrate the cartridge with 10 column volumes of buffer.**  
Use a flow rate of 1 ml/min (1 ml cartridges) or 5 ml/min (5 ml cartridges).
- 4. Using the system pumps or a superloop load the cleared cell lysate onto the cartridge.**  
To ensure that the target protein has bound to the *Strep*-Tactin resin, retain the flow-through fraction for analysis by SDS-PAGE.

**5. Fill system pumps with Buffer NP and wash the cartridge until the A280 returns to the baseline value.**

Retain the wash fraction for analysis by SDS-PAGE.

**6. Fill system pumps with Buffer NPD and elute protein from cartridge.**

Protein usually elutes within 5–10 column volumes. A shallow linear gradient over 20 column volumes may help separate proteins. *Strep*-Tactin Superflow Plus Cartridges can be regenerated using the procedure provided in Appendix C on page 57.

# Protocol: Batch Purification of *Strep*-tagged Proteins Using *Strep*-Tactin Superflow Plus

The composition of the lysis and elution buffers can be modified to suit the particular application, e.g., by adding Tween, DTT, or PMSF, or increasing NaCl or glycerol concentrations. See Table 2 on page 12 for a list of buffer reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction.

## Important points before starting

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

## Equipment and reagents to be supplied by user

- Cleared lysate
- *Strep*-Tactin Superflow Lysis Buffer (Buffer NP)
- Empty columns
- *Strep*-Tactin Superflow Elution Buffer (Buffer NPD)
- *Strep*-Tactin Superflow Plus

Buffer compositions are provided in Appendix B on page 52.

## Procedure

- 1. Pipet cleared cell lysate into a 15 ml tube and add 2 ml *Strep*-Tactin Superflow Plus Resin suspension. Mix gently by shaking (200 rpm on a rotary shaker) at 4°C for 60 min.**
- 2. Load the *Strep*-Tactin Superflow resin into a column with the bottom outlet capped.**
- 3. Remove bottom cap and collect the flow-through.**  
Save flow-through for SDS-PAGE analysis.
- 4. Wash column two times with 4 ml Buffer NP.**  
Collect wash fractions for SDS-PAGE analysis.
- 5. Elute the protein six times with 0.5 ml Buffer NPD. Collect eluates in six tubes and analyze by SDS-PAGE.**  
*Strep*-Tactin Superflow Plus can be regenerated using the procedure provided in Appendix C on page 57.

## Protocol: Micro-Scale Purification of *Strep*-tagged Proteins Using *Strep*-Tactin Magnetic Beads

The composition of the lysis and elution buffers can be modified to suit the particular application, for example by adding Tween, DTT, or PMSF, or increasing NaCl or glycerol concentrations. See Table 2 on page 12 for a list of buffer reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction.

The amount of tagged protein recovered using *Strep*-Tactin Magnetic Beads is proportional to the protein concentration in the sample. Therefore, if samples contain low concentrations of protein, we recommended removing the storage buffer from the *Strep*-Tactin Magnetic Beads before adding the sample to the beads.

Purification using *Strep*-Tactin Magnetic Beads can also be performed in 96-well plate format. Adjust volumes given in the protocol according to the total volume used, incubate on a horizontal shaker platform, and use a 96-well Magnet (Type A; QIAGEN cat. no. 36915) to deposit beads.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Cleared lysate
- *Strep*-Tactin Magnetic Beads
- *Strep*-Tactin Beads Lysis Buffer (Buffer NP-T)
- *Strep*-Tactin Beads Elution Buffer (Buffer NPB-T)
- Optional: Benzonase Nuclease

Buffer compositions are provided in Appendix B on page 52.

## Procedure

- 1. Resuspend *Strep*-Tactin Magnetic Beads by vortexing for 2 s and then immediately add 200  $\mu$ l of 10% *Strep*-Tactin Magnetic Bead suspension to a cleared lysate or the pooled fractions eluted from Ni-NTA Magnetic Beads.**

**Note:** Care is necessary to ensure that constant amounts of beads are pipetted. The beads will settle if the suspension is not agitated regularly. 200  $\mu$ l of *Strep*-Tactin Magnetic Beads suspension has a binding capacity of 40–60  $\mu$ g protein (see Table 1, page 11). If significantly different amounts of tagged protein are present in your lysate, the volume of magnetic-bead suspension should be varied accordingly. However, use of volumes less than 10  $\mu$ l are not recommended due to the associated handling problems — smaller volumes are difficult to pipet and may lead to uneven distribution of beads and reduced reproducibility. Generally, we recommend using cleared lysates for binding to *Strep*-Tactin Magnetic Beads. However, it may be possible to obtain good results by using crude lysates without clearing them. In this case, add Benzonase Nuclease (3 U per ml culture volume), and incubate on ice for 10–15 min.

- 2. Mix the suspension gently on an end-over-end shaker for 30–60 min at 4°C.**
- 3. Place the tube on a magnetic separator for 1 min and remove supernatant with a pipet.**

Tubes may be briefly centrifuged, before placing on the magnetic separator, to collect droplets of suspension from the tube caps.

- 4. Remove the tube from the magnet, add 500  $\mu$ l Buffer NP-T, gently vortex the suspension, place the tube on a magnetic separator for 1 min, and remove buffer.**
- 5. Repeat step 4.**  
Remaining buffer should be removed completely.

- 6. Add 50  $\mu$ l Buffer NPB-T, gently vortex the suspension, incubate the tube for 5 min, place the tube on a magnetic separator for 1 min, and collect the eluate in a clean tube.**

Tubes may be centrifuged before placing on the magnetic separator, to collect droplets of suspension from the tube caps.

- 7. Repeat step 6 three times to give four eluate fractions.**

Because the biotin used for elution binds *Strep*-Tactin with extremely high affinity, it is not possible to regenerate *Strep*-Tactin Magnetic Beads.

## Protocol: Immunodetection Using the *Strep*-tag Antibody (Chemiluminescent Method)

This protocol is used for chemiluminescent detection of *Strep*-tagged proteins on western or dot blots.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Western blot or dot blot
- TBS (10 mM Tris·Cl; 150 mM NaCl, pH 7.5)
- TBS-Tween/Triton (20 mM Tris·Cl; 500 mM NaCl; 0.05% (v/v) Tween; 0.2% (v/v) Triton, pH 7.5)
- Blocking buffer (3% BSA or 1% casein in TBS)
- *Strep*-tag Antibody stock solution: Dissolve the lyophilized *Strep*-tag Antibody in 500  $\mu$ l water. The reconstituted solution contains 200  $\mu$ g/ml *Strep*-tag Antibody in PBS with PEG, sucrose, and sodium azide (0.08% [w/v]).
- Anti-mouse secondary antibody conjugate
- Secondary antibody dilution buffer (10% milk powder or 1% casein in TBS)

For compositions of buffers and reagents, see Appendix B on page 52.

The solutions required depend on the detection method employed.

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. Alternatively, if alkali-soluble casein (Merck, cat. no. 1.02241) is available in your country it can be used as a blocking reagent throughout the chemiluminescent detection protocol. The reagents used are shown in Table 4.

**Table 4. Reagents used in chemiluminescent detection of *Strep*-tagged proteins**

<b>Step</b>		<b>(Alternative method)</b>
Blocking	3% BSA in TBS	1% casein in TBS
<i>Strep</i> -tag Antibody binding	3% BSA in TBS	1% casein in TBS
Secondary antibody binding	10% milk powder in TBS	1% casein in TBS

### **Chemiluminescent substrates**

CDP-Star™ can be used with AP-conjugated secondary antibodies, and the ECL system can be used in combination with HRP-conjugated secondary antibodies.

### **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 (15–25°C).**
- 5. Incubate with *Strep*-tag Antibody (1/1000–1/2000 dilution of antibody stock solution in blocking buffer) at room temperature (15–25°C) for 1 h.**  
Membrane can be sealed in plastic bags. 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.**

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

**8. Incubate with secondary antibody solution for 1 h at room temperature (15–25°C).**

Either alkaline phosphatase (AP) or HRP conjugated anti-mouse IgG may be used. 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 can be used for this protocol step.

# Protocol: Immunodetection Using the Strep-tag Antibody (Chromogenic Method)

The solutions required depend on the detection method employed.

## Important points before starting

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

## Equipment and reagents to be supplied by user

- Western blot, dot blot, or colony blot
- TBS (10 mM Tris·Cl; 150 mM NaCl, pH 7.5)
- TBS-Tween/Triton (20 mM Tris·Cl; 500 mM NaCl; 0.05% (v/v) Tween; 0.2% (v/v) Triton, pH 7.5)
- Strep-tag Antibody
- Anti-mouse secondary antibody AP or HRP conjugate
- Blocking buffer (3% BSA in TBS)
- Secondary antibody dilution buffer (3% BSA in TBS)
- Staining solutions for AP or HRP

The solutions required depend on the antibody and detection method used. For the chromogenic detection method, 3% (w/v) BSA in TBS is used as a blocking reagent throughout the whole procedure.

## 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 (15–25°C).**  
3% BSA (w/v) in TBS buffer is used for blocking throughout the procedure when using chromogenic detection.
- 3. Wash membrane twice for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**  
Use of TBS-Tween/Triton buffer has been found empirically to result in optimal signal-to-noise ratios.
- 4. Wash membrane for 10 min with TBS buffer at room temperature (15–25°C).**

- 5. Incubate with *Strep*-tag Antibody (1/1000–1/2000 dilution of antibody stock solution in blocking buffer) at room temperature for 1 h.**

Membrane can be sealed in plastic bags.

3% BSA (w/v) in TBS buffer, is used for blocking throughout the procedure when using chromogenic 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.**
- 8. Incubate with secondary antibody solution diluted in 3% BSA (w/v) in TBS for 1h at room temperature (15–25°C).**

Either AP- or 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. Dilute according to the manufacturer's recommendations. Use the lowest recommended amounts to avoid false signals.

- 9. Wash four times for 10 min each time in TBS-Tween/Triton buffer at room temperature (15–25°C).**

#### Detection

- 10. Stain with AP or HRP staining solution until the signal is clearly visible (approximately 5–15 min).**

Do not shake blots during color development.

- 11. Stop the chromogenic reaction by rinsing the membrane twice with water.**
- 12. Dry the membrane and photograph as soon as possible.**
- 13. The colors will fade with time. The product formed when using HRP is particularly unstable.**

## Troubleshooting Guide

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

### Comments and suggestions

---

#### Purification using *Strep-Tactin* matrices

##### Protein does not bind to the *Strep-Tactin* matrix

- |  |   |
|--|---|
| a) Binding conditions are incorrect                                | Avoid using chaotropic salts in buffers. In contrast to 6xHis-tag–Ni-NTA interaction even low (<500 mM) concentrations of urea will disturb the <i>Strep</i> -tag II– <i>Strep-Tactin</i> interaction. Check buffer pH, which should be above 6.5.                                      |
| b) <i>Strep-Tactin</i> matrix binding sites are occupied/blocked   | Biotin irreversibly blocks the binding sites of <i>Strep-Tactin</i> and should be removed from protein samples by dialysis; alternatively avidin can be added to protein samples. Addition of avidin does not interfere with the <i>Strep</i> -tag II– <i>Strep-Tactin</i> interaction. |
| c) <i>Strep</i> -tag is not present                                | Sequence ligation junctions to ensure that the reading frame is correct.<br><br>Check for possible internal translation starts (N-terminal tag) or premature termination sites (C-terminal tag).  |
| d) <i>Strep</i> -tag is inaccessible                               | Move tag to the opposite end of the protein.  |
| e) <i>Strep</i> -tag– <i>Strep-Tactin</i> interaction is very weak | Replace batch purification by applying the lysate to a prepared column.<br><br>Use more concentrated lysate or Ni-NTA eluate and remove <i>Strep-Tactin</i> Magnetic Beads storage buffer before use.   |

## Comments and suggestions

---

- f) Protein does not elute from *Strep*-Tactin resin      The *Strep*-tag II–*Strep*-Tactin interaction has a  $K_D$  value in the  $\mu\text{M}$  range. Therefore, tight binding is most probably caused by unspecific binding to the Superflow or Magnetic Agarose Beads matrix.  
Elution with 10 mM biotin is possible, but all *Strep*-Tactin binding sites will be blocked irreversibly.
- g) Protein has precipitated on the column      Add detergents to or reduce the salt concentration in buffers. Do not use a pH near the pI of the protein. Increase or lower pH (pH must be above 6.5).

### Purification using Ni-NTA matrices

#### Protein does not bind to the Ni-NTA resin

- 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      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 and composition of all buffers and solutions.  
Ensure that there are no chelating or reducing agents present, and that the concentration of imidazole is not too high.
- e) Protein elutes in the wash buffer      Wash stringency is too high. Lower the concentration of imidazole or increase the pH slightly.
- f) 6xHis tag is partially hidden      Reduce wash stringency.
- g) Buffer conditions incorrect      Check pH and composition of wash buffer.  
Ensure that there are no chelating or reducing agents present.

## Comments and suggestions

---

- h) Protein precipitates during purification      Temperature is too low. Perform purification at room temperature (15–25°C).
- i) Protein forms aggregates      Try adding solubilization reagents such as 0.1% Triton X-100 or Tween-20, up to 20 mM  $\beta$ -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) Elution conditions are too mild (protein may be in an aggregate or multimer form)      Elute with a pH or imidazole step gradient to determine the optimal elution conditions.
- b) Protein has precipitated on the column      Perform binding and elution in batch format to avoid high local protein 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  $\beta$ -ME to a maximum of 20 mM or DTT to 10 mM to reduce disulfide bonds.  
Increase salt and/or detergent concentrations, or add ethanol/glycerol to wash buffer to disrupt nonspecific interactions.
- c) Contaminants are associated with tagged protein      Check for possible internal translation starts (C-terminal tag) or premature termination sites (N-terminal tag).

### Discoloration of resin

- Nickel ions are removed or reduced      Ensure that there are no chelating compounds (resin turns white in color) or high concentrations of reducing agents (resin turns brown in color) present in the buffers.

## Comments and suggestions

---

### Purification from mammalian cells

- a) No protein band in SDS-PAGE analysis of the eluate
- Expression is too low. Check the expression level by western blotting using an Anti-His Antibody or a protein-specific antibody. Alternatively, perform an immunoassay with Ni-NTA Magnetic Agarose Beads (see the *Ni-NTA Magnetic Agarose Beads Handbook*) or ELISA using Ni-NTA HisSorb Strips (see the *QIAexpress<sup>®</sup> Detection and Assay Handbook*). If only small amounts of 6xHis-tagged protein are present in the lysate, increase the amount of starting cell material and purify with an equal amount of magnetic beads. Do not exceed lysis volumes of 2 ml — this allows purification in a single 2 ml tube.
- b) 6xHis tagged protein has been degraded
- Check that the 6xHis tag is not removed from the protein during post-translational processing. Work at 4°C and add protease inhibitors, such as PMSF.
- c) 6xHis-tagged protein partially elutes in the wash
- The binding capacity used is too low to bind all of the 6xHis-tagged protein. A 10  $\mu$ l magnetic-bead suspension has a binding capacity of 3  $\mu$ g 6xHis-tagged DHFR (24 kDa). If significantly larger amounts of 6xHis-tagged protein are present in the lysate, increase the amount of beads accordingly.

## Comments and suggestions

---

### Binding of contaminants

- a) Too much Ni-NTA matrix was used
- Match the total binding capacity of the beads to the amount of 6xHis-tagged protein to be purified by simply adjusting the amount of Ni-NTA Magnetic Agarose Beads suspension used.
- Proteins that contain neighboring histidines are not common in bacteria, but do occur in eukaryotic cells. These proteins, as well as endogenous proteins with metal-binding sites, normally bind with lower affinity to the Ni-NTA matrix than do 6xHis-tagged proteins. If the binding capacity of the amount of beads used greatly exceeds the amount of 6xHis-tagged protein to be purified, these proteins will bind to the Ni-NTA matrix to a considerably higher extent, and will be subsequently recovered in the eluate.
- b) Binding and wash conditions are not stringent enough
- Always include 10–20 mM imidazole in the binding buffer and 20 mM imidazole in the wash buffer.
- c) Large amount of nontagged proteins in the lysate when purifying from cells with a low expression rate
- Perform a second round of purification from the eluate after adjusting the imidazole concentration to 10–20 mM using binding buffer without imidazole. Significantly smaller amounts of background proteins in the binding step reduce the level of contaminants in the final preparation.

---

### Purification from insect cells

#### No protein band in SDS-PAGE analysis of the fractions

- a) No or low expression
- Check the expression level by western blotting using an Anti-His Antibody or a protein-specific antibody. Alternatively perform an ELISA using Ni-NTA HisSorb Strips (see *QIAexpress Detection and Assay Handbook*). If low amounts of 6xHis-tagged protein are present in the lysate, increase the amount of starting cell material and purify with an equal amount of Ni-NTA matrix.

## Comments and suggestions

---

- |   |  |
|---|--|
| b) 6xHis tagged protein has been degraded                                   | Check that the 6xHis tag is not removed from the protein during post-translational processing or by endogenous proteases during the purification procedure.<br><br>Work at 4°C and add protease inhibitors, such as PMSF.                |
| c) 6xHis tagged protein partially elutes in the wash buffer or flow-through | The amount of matrix used is too low to bind all of the 6xHis-tagged protein. 100 $\mu$ l Ni-NTA agarose has a binding capacity of 500–1000 $\mu$ g 6xHis-tagged protein. Adjust the amount of matrix used for purification accordingly. |
- 

## Contaminants bind to resin

- |   |   |
|---|---|
| a) Too much Ni-NTA matrix was used                      | Match the total binding capacity of the matrix used to the amount of 6xHis-tagged protein to be purified. Endogenous proteins with metal-binding sites normally bind with lower affinity to the Ni-NTA matrix than do 6xHis-tagged proteins. If the binding capacity of the amount of matrix used greatly exceeds the amount of 6xHis-tagged protein to be purified, these proteins will bind to the Ni-NTA matrix to a considerably higher extent, and subsequently will be recovered in the eluate. |
| b) Binding and wash conditions are not stringent enough | Always include 10–20 mM imidazole in the binding buffer and 20 mM imidazole in the wash buffer.   |

## Appendix A: Two-Step Purification Using a His-tag and a Strep-tag

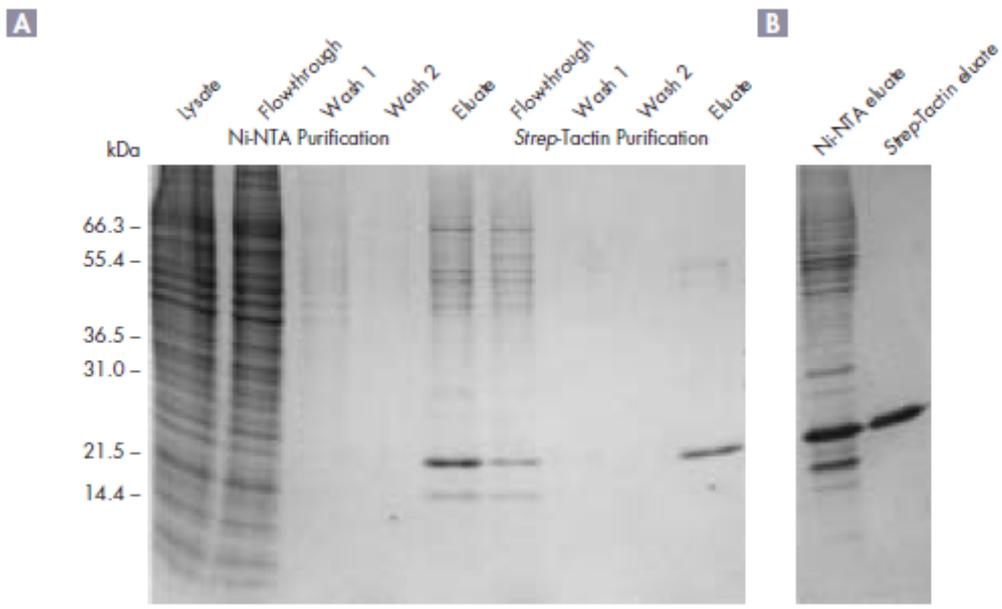
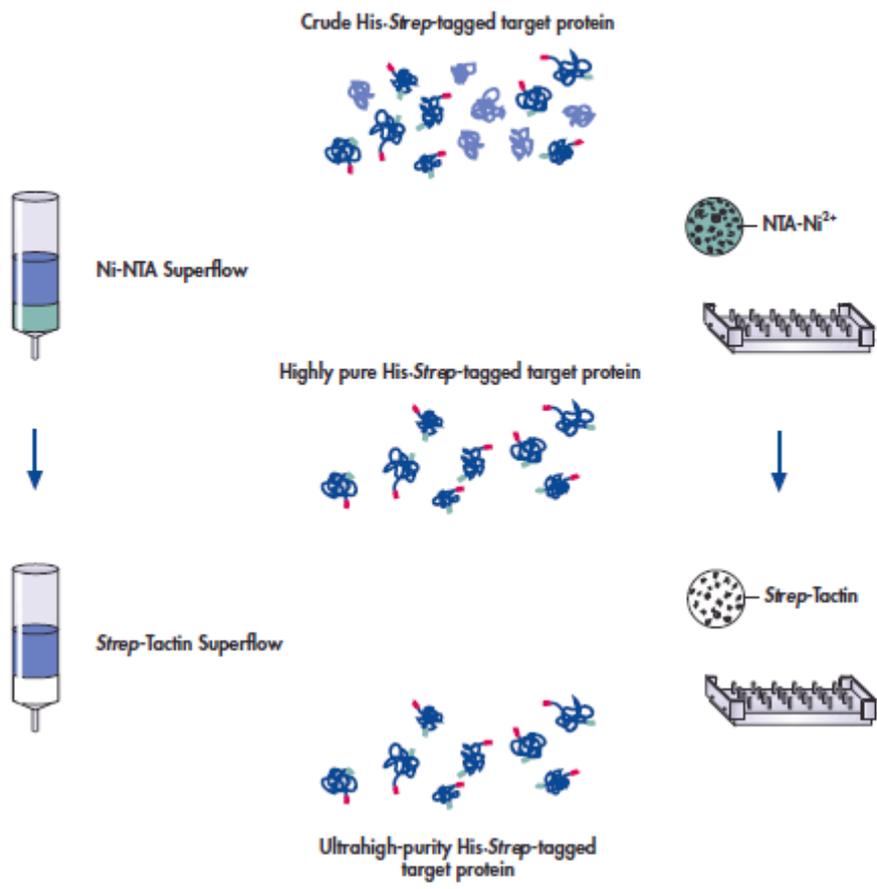
Many researchers in structural and functional proteomics use eukaryotic systems, such as insect or mammalian cells, to obtain protein post-translational modifications not provided by conventional bacterial expression. However, the complexity of eukaryotic proteomes makes purification of proteins expressed using these systems a challenge and typically means that individual purification schemes must be developed for each protein. There is therefore an urgent need for a standardized method for purification of functional, high-purity proteins. In order to meet this need, QIAGEN offers a two-step affinity purification process, which provides ultrapure protein even from eukaryotic expression systems. To increase purity, proteins can be tagged with both a His- and *Strep*-tag and purified in a two-step procedure using Ni-NTA and *Strep*-Tactin. Recombinant proteins that carry the two small affinity tags are efficiently expressed in *E. coli*, insect, or mammalian cells using pQE-TriSystem His·*Strep* vectors (Figure 5, page 41). After cell lysis and clearing of the lysate, proteins are initially purified using an immobilized-metal affinity chromatography procedure that is based on the proven 6xHis-tag–Ni-NTA interaction. After elution from the Ni-NTA matrix using imidazole, recombinant proteins (which also carry the *Strep*-tag II epitope) are loaded directly onto a *Strep*-Tactin matrix (see flowchart, page 40). No buffer exchange is required. Protein is eluted from the *Strep*-Tactin matrix using either biotin or desthiobiotin. This two-step affinity purification delivers ultrapure (>98% pure) protein (Figure 4, page 34). The order of purifications can be reversed (i.e., *Strep*-Tactin followed by Ni-NTA purification).

### Initial purification of His·*Strep*-tagged proteins using the 6xHis-tag–Ni-NTA interaction

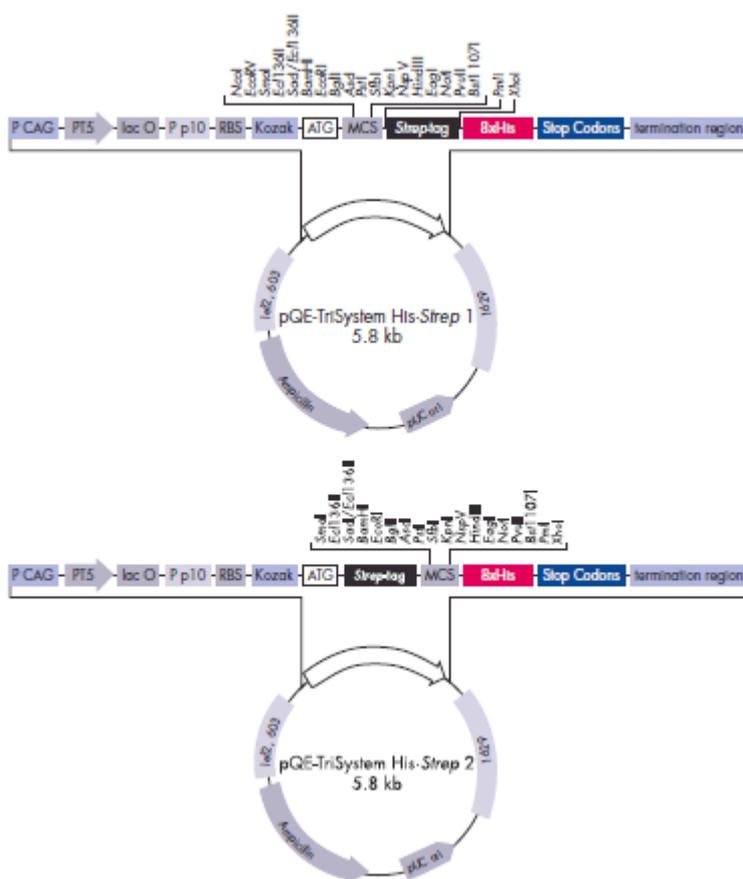
The initial stage of His·*Strep*-tagged protein purification is based on the remarkable selectivity and high affinity of patented Ni-NTA (nickel-nitrilotriacetic acid) resin for proteins containing an affinity tag of six consecutive histidine residues — the 6xHis tag. NTA, which has four chelation sites for nickel ions, binds nickel more tightly than metal-chelating purification systems that only have three sites available for interaction with metal ions. The extra chelation site prevents nickel-ion leaching; providing a greater binding capacity, and high-purity protein preparations.

**Table 5. Protein binding capacities of Ni-NTA matrices**

Matrix	Protein binding capacity
Ni-NTA Superflow and Agarose	Up to 50 mg/ml
Ni-NTA Magnetic Agarose Beads	Up to 2 mg/ml (5% suspension)



**Figure 4. Thioredoxin, expressed in NIH-3T3 cells, was purified using the Two-Step Affinity Purification System. **A** Coomassie<sup>®</sup>-stained gel. **B** Silver-stained gel.**



**Figure 5. pQE-TriSystem His-Strep vectors for parallel protein expression using a single construct in *E. coli*, insect, and mammalian cells.** PT5: T5 promoter; lac O: lac operator; RBS: Ribosome binding site; ATG: Start codon; 8xHis: His tag sequence, MCS: Multiple cloning site; Strep-tag: Strep-tag sequence; Stop Codons: Stop codons in all three reading frames; Ampicillin: Ampicillin resistance gene; P CAG: CMV/actin/globin promoter; P p10: p10 promoter; Kozak: Kozak consensus sequence; termination region: Transcription terminator region; lef2, 603/1629: Flanking baculovirus sequences to permit generation of recombinant baculoviruses; pUC ori: pUC origin of replication.

Some reagents and buffer components used for protein purification may interfere with the affinity interaction between the protein and purification matrix. Table 6 lists compatibility of various reagents with the Ni-NTA–6xHis interaction.

**Table 6. Reagents compatible with the 6xHis/Ni-NTA interaction\***

<b>Denaturants</b>	<b>Detergents</b>	<b>Reducing agents</b>	<b>Others</b>	<b>Salts</b>	<b>For long-term storage</b>
6 M Gu-HCl	2% Triton X-100	20 mM $\beta$ -ME	50% Glycerol	4 M MgCl <sub>2</sub>	Up to 30% ethanol
8 M Urea	2% Tween 20	10 mM DTT	20% Ethanol	5 mM CaCl <sub>2</sub>	100 mM NaOH
	1% CHAPS	20 mM TCEP	20 mM imidazole <sup>†</sup>	2 mM NaCl	
				1 mM PMSF	

\* The reagents listed have been successfully used in concentrations up to those given. A more comprehensive list of reagents compatible with the His-tag–Ni-NTA interaction can be found in *The QIAexpressionist™*.

<sup>†</sup> Higher concentrations of imidazole are used to elute His-tagged proteins.

## Protocol: Protein Purification from Insect-Cell Growth Medium

To purify proteins secreted into insect-cell growth medium, the pH and salt concentrations of the medium must be adjusted to provide optimal conditions for protein binding to Ni-NTA. However, media used to culture insect cells may contain electron-donating groups (e.g., the amino acids glutamine, glycine, or histidine) that can prevent binding of 6xHis tagged proteins to Ni-NTA. If purification fails, dialysis, ultrafiltration, or gel filtration can be used to adjust the sample to the optimal buffer conditions for Ni-NTA purification.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Insect-cell growth medium containing secreted proteins
- 10x Binding Buffer (500 mM NaH<sub>2</sub>PO<sub>4</sub>; 1.5 M NaCl, pH 8.0)

Buffer compositions are provided in Appendix B on page 52.

### Procedure

- 1. Add a volume of the 10x binding buffer to the insect-cell growth medium that will result in a 1x end concentration and mix.**

For instance, if you have 45 ml insect-cell medium, add 5 ml 10x binding buffer to give a total volume of 50 ml.

- 2. Proceed using one of the Ni-NTA purification protocols.**

## Protocol: Manual Purification of His-tagged Proteins Using a Syringe

This protocol is for purification of His-tagged proteins using Ni-NTA Superflow Cartridges and a syringe. The composition of the lysis, wash, and elution buffers can be modified to suit the particular application, for example by adding Tween,  $\beta$ -mercaptoethanol, or PMSF, or increasing NaCl or glycerol concentrations. See Table 6 on page 42 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

### Important points before starting

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

### Things to do before starting

- Immediately before applying to column, sterile filter (0.2 or 0.45  $\mu\text{m}$ ) or centrifuge the lysate (10,000  $\times$  g) to ensure that it is particle-free.

### Equipment and reagents to be supplied by user

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

Buffer compositions are provided in Appendix B on page 52.

### Procedure

- 1. Fill syringe with Buffer NPI-10 (native conditions) or Buffer B (denaturing conditions), attach a suitable adapter, and expel air by depressing syringe plunger until buffer drips from the end of the adapter.**
- 2. Attach syringe to the cartridge inlet and remove cartridge outlet stopper.**
- 3. Equilibrate the cartridge with 10 column volumes of buffer.**

Do not use high pressure to force buffer through the cartridge. Ideal flow rates are 1 ml/min (1 ml cartridges) and 5 ml/min (5 ml cartridges).

- 4. Remove syringe and fill with cleared lysate. Apply cleared lysate to the cartridge using the same flow rate as in step 3.**
- 5. Using a fresh syringe and the same flow rate as in steps 3 and 4, wash the cartridge with 10 column volumes of Buffer NPI-20 (native conditions) or Buffer C (denaturing conditions).**
- 6. Fill a syringe with Buffer-NPI 250 (native conditions) or Buffer E (denaturing conditions) and elute protein from cartridge using the recommended flow rate.**

Protein usually elutes within 5–10 column volumes.

## Protocol: Purification of His-tagged Proteins Using Liquid Chromatography Systems

This protocol is suitable for purifying His-tagged proteins using Ni-NTA Superflow Cartridges on liquid chromatography systems (such as the ÄKTA or FPLC System). During equilibration, loading, washing, and elution monitor the back pressure generated by the chromatography system. Do not allow back pressure to exceed 5 bar (0.5 MPa). The composition of the lysis, wash, and elution buffers can be modified to suit the particular application, for example by adding Tween,  $\beta$ -mercaptoethanol, or PMSF, or increasing NaCl or glycerol concentrations. See Table 6 on page 42 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

### Important points before starting

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

### Things to do before starting

- Immediately before applying to column, sterile filter (0.2 or 0.45  $\mu\text{m}$ ) or centrifuge the lysate (10,000  $\times g$ ) to ensure that it is particle-free.

### Equipment and reagents to be supplied by user

- Cleared cell lysate containing 6xHis-tagged protein
- Buffers NPI-10, NPI-20, and NPI-250 (native conditions) or Buffers B, C, and E (denaturing conditions). All buffers should be sterile filtered (0.2 or 0.45  $\mu\text{m}$ ) before use.
- Connector adapter

Buffer compositions are provided in Appendix B on page 52.

### Procedure

- 1. Fill system pumps with Buffer NPI-10 (native conditions) or Buffer B (denaturing conditions) and attach cartridge to the pump outlet, taking care not to introduce air into the system.**
- 2. Remove cartridge outlet stopper and attach to the system tubing.**
- 3. Equilibrate the cartridge with 10 column volumes of buffer.**  
Use a flow rate of 1 ml/min (1 ml cartridges) or 5 ml/min (5 ml cartridges).
- 4. Using the system pumps or a superloop load the cleared cell lysate onto the cartridge.**

To ensure that the target protein has bound to the Ni-NTA resin, retain the flow-through fraction for analysis by SDS-PAGE.

- 5. Fill system pumps with Buffer NPI-20 (native conditions) or Buffer C (denaturing conditions) and wash the cartridge until the A280 returns to the baseline value.**

Retain the wash fraction for analysis by SDS-PAGE.

- 6. Fill system pumps with Buffer-NPI 250 (native conditions) or Buffer E (denaturing conditions) and elute protein from cartridge.**

Protein usually elutes within 5–10 column volumes. A shallow linear gradient over 20 column volumes may help separate proteins.

## Protocol: Batch Purification of His·Strep-tagged Proteins Using Ni-NTA Matrices

Ni-NTA Resin Lysis Buffer contains 10 mM imidazole to minimize binding of untagged, contaminating proteins and increase purity with fewer wash steps. If the tagged protein does not bind under these conditions, the amount of imidazole should be reduced to 1–5 mM. With His·Strep-tagged proteins exhibiting high binding affinities, the imidazole concentration can be increased to 20 mM.

The composition of the lysis, wash, and elution buffers can be modified to suit the particular application, for example by adding Tween,  $\beta$ -mercaptoethanol, or PMSF, or increasing NaCl or glycerol concentrations. See Table 6 on page 42 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Cleared cell lysate
- Buffers NPI-10 and NPI-20 (native conditions) or Buffers C and E (denaturing conditions). All buffers should be sterile filtered (0.2 or 0.45  $\mu$ m) before use.
- Ni-NTA Superflow or Ni-NTA Agarose
- Empty columns

Buffer compositions are provided in Appendix B on page 52.

### Procedure

- 1. Add 1 ml of 50% Ni-NTA matrix slurry to 4 ml cleared lysate and mix gently by shaking (200 rpm on a rotary shaker) at 4°C for 60 min.**
- 2. Load the lysate–Ni-NTA mixture into a column with the bottom outlet capped.**
- 3. Remove bottom cap and collect the column flow-through.**  
Save flow-through for SDS-PAGE analysis.
- 4. Wash twice with 4 ml Buffer NPI-20 (native conditions) or Buffer C (denaturing conditions).**  
Collect wash fractions for SDS-PAGE analysis.

- 5. Elute the protein four times with 0.5 ml Buffer-NPI 250 (native conditions) or Buffer E (denaturing conditions). Collect the eluate in four tubes and analyze by SDS-PAGE.**

## Protocol: Micro-Scale Purification of His·Strep-tagged Proteins Using Ni-NTA Magnetic Agarose Beads

The composition of the lysis, wash, and elution buffers can be modified to suit the particular application, for example by adding Tween,  $\beta$ -mercaptoethanol, or PMSF, or increasing NaCl or glycerol concentrations. See Table 6 on page 42 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

Purification using Ni-NTA Magnetic Agarose Beads can also be performed in 96-well plate format. Adjust volumes given in the protocol according to the total volume used, incubate on a horizontal shaker platform, and use a 96-well Magnet (Type A; QIAGEN cat. no. 36915) to deposit beads.

### Important points before starting

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

### Equipment and reagents to be supplied by user

- Cleared cell lysate
- Buffers NPI-20-T and NPI- (native conditions) or Buffers C and E (denaturing conditions)
- **Optional:** Benzonase Nuclease

Buffer compositions are provided in Appendix B on page 52.

### Procedure

- 1. Resuspend the Ni-NTA Magnetic Agarose Beads by vortexing for 2 s and then immediately add 200  $\mu$ l of the 5% Ni-NTA Magnetic Agarose Bead suspension to each 1 ml of lysate containing the His·Strep-tagged protein.**

**Note:** Care is necessary to ensure that constant amounts of beads are pipetted. The beads will settle if the suspension is not agitated regularly. 200  $\mu$ l magnetic bead suspension has a binding capacity of 60  $\mu$ g 6xHis-tagged DHFR (24 kDa). If significantly different amounts of tagged protein are present in your lysate, the volume of magnetic-bead suspension should be varied accordingly. However, use of volumes less than 10  $\mu$ l are not recommended due to the associated handling problems — smaller volumes are difficult to pipet and may lead to uneven distribution of beads and reduced reproducibility. Generally, we recommend using cleared lysates for binding to Ni-NTA Magnetic Agarose Beads. However, it may be possible to

obtain good results by using crude lysates without clearing them. In this case, use lysates which have been diluted 5-fold, and if using native lysis conditions, add Benzonase Nuclease (3 U per ml culture volume), and incubate on ice for 10–15 min.

**2. Mix the suspension gently on an end-over-end shaker for 30–60 min at room temperature (15–25°C).**

The time and temperature necessary for efficient binding is dependent on the protein and the accessibility of the 6xHis tag in the buffer system used. Especially under native conditions, it may be necessary to incubate at 4°C if the protein is not stable at room temperature.

**3. Place the tube on a magnetic separator for 1 min and remove supernatant with a pipet.**

Tubes may be briefly centrifuged, before placing on the magnetic separator, to collect droplets of suspension from the tube caps.

**4. Remove tube from the magnet, add 500 µl of Buffer NPI-20-T (native conditions) or Buffer C (denaturing conditions), mix the suspension, place the tube on a magnetic separator for 1 min, and remove buffer.**

**5. Repeat step 4.**

Remaining buffer should be removed completely.

**6. Add 100 µl of Buffer NPI-250-T (native conditions) or Buffer E (denaturing conditions), mix the suspension, incubate the tube for 1 min, place for 1 min on a magnetic separator, and collect the eluate in a clean tube.**

Tubes may be centrifuged before placing on the magnetic separator, to collect droplets of suspension from the tube caps.

**7. Repeat step 6.**

Most of the 6xHis-tagged protein will elute in the first elution step. If a more concentrated protein solution is required, elute in two aliquots of 50 µl.

## Appendix B: Buffer Compositions

### Bacterial media

LB medium 10 g/liter tryptone; 5 g/liter yeast extract;  
10 g/liter NaCl

### 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

### 10x Binding Buffer (1 liter)

500 mM NaH<sub>2</sub>PO<sub>4</sub> 69.0 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

1.5 M NaCl 87.7 g NaCl (MW 58.44 g/mol)

Adjust pH to 8.0 using NaOH.

### Buffers for purification of proteins using *Strep-Tactin Superflow Plus*

#### Buffer NP\* (1 liter)

50 mM NaH<sub>2</sub>PO<sub>4</sub> 6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)

Adjust pH to 8.0 using NaOH.

#### Buffer NPD (1 liter)

50 mM NaH<sub>2</sub>PO<sub>4</sub> 6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)

2.5 mM desthiobiotin 0.54 g desthiobiotin (Sigma cat. no. D 1411)

Adjust pH to 8.0 using NaOH.

\* Lysis buffers for insect cells should be supplemented with 1% Igepal CA-630.

## Buffers for purification of proteins using Strep-Tactin Magnetic Beads

### Buffer NP-T (1 liter)

50 mM NaH <sub>2</sub> PO <sub>4</sub>	6.90 g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
0.05% Tween 20	5 ml of a 10% Tween 20 stock solution
Adjust pH to 8.0 using NaOH.	

### Buffer NPB-T (1 liter)

50 mM NaH <sub>2</sub> PO <sub>4</sub>	6.90 g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
10 mM biotin	2.44 g biotin (Sigma cat. no. B 4501)
0.05% Tween 20	5 ml of a 10% Tween 20 stock solution
Adjust pH to 8.0 using NaOH.	

### Strep-Tactin Superflow Regeneration Buffer (1 liter)

50 mM NaH <sub>2</sub> PO <sub>4</sub>	6.90 g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
1 mM HABA	0.24 g HABA (Sigma cat. no. H 5126)
Adjust pH to 8.0 using NaOH.	

## Buffers for purification of proteins using Ni-NTA Superflow

### Buffer NPI-10\* (1 liter)

50 mM NaH <sub>2</sub> PO <sub>4</sub>	6.90 g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
10 mM imidazole	0.68 g imidazole (MW 68.08 g/mol)
Adjust pH to 8.0 using NaOH.	

\* Lysis buffers for insect cells should be supplemented with 1% Igepal CA-630.

**Buffer NPI-20 (1 liter)**

50 mM NaH<sub>2</sub>PO<sub>4</sub>                      6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl                              17.54 g NaCl (MW 58.44 g/mol)

20 mM imidazole                      1.36 g imidazole (MW 68.08 g/mol)

Adjust pH to 8.0 using NaOH.

**Buffers for purification of proteins using Ni-NTA Superflow****Buffer NPI-250 (1 liter)**

50 mM NaH<sub>2</sub>PO<sub>4</sub>                      6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl                              17.54 g NaCl (MW 58.44 g/mol)

250 mM imidazole                      17.00 g imidazole (MW 68.08 g/mol)

Adjust pH to 8.0 using NaOH.

**Buffer B (1 liter)**

8M Urea                                      480.50 g urea (MW 60.06 g/mol)

100 mM NaH<sub>2</sub>PO<sub>4</sub>                      13.80 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

10 mM Tris·Cl                              12.10 g Tris·Cl (MW 121.10 g/mol)

Adjust pH to 8.0 using HCl.

**Buffer C (1 liter)**

8M Urea                                      480.50 g urea (MW 60.06 g/mol)

100 mM NaH<sub>2</sub>PO<sub>4</sub>                      13.80 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

10 mM Tris·Cl                              12.10 g Tris·Cl (MW 121.10 g/mol)

Adjust pH to 6.3 using HCl.

**Buffer E (1 liter)**

8M Urea                                      480.50 g urea (MW 60.06 g/mol)

100 mM NaH<sub>2</sub>PO<sub>4</sub>                      13.80 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

10 mM Tris·Cl                              12.10 g Tris·Cl (MW 121.10 g/mol)

Adjust pH to 4.5 using HCl.

## Buffers for purification of proteins using Ni-NTA Magnetic Agarose Beads

### PBS (1 liter)

50 mM N NaH<sub>2</sub>PO<sub>4</sub> 6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

150 mM NaCl 8.77 g NaCl (MW 58.44 g/mol)

Adjust pH to 7.2 using NaOH.

### Buffer NPI-10-T (1 liter):

50 mM NaH<sub>2</sub>PO<sub>4</sub> 6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)

10 mM imidazole 0.68 g imidazole (MW 68.08 g/mol)

0.05% Tween 20 5 ml of a 10% Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

### Buffer NPI-20-T (1 liter)

50 mM N NaH<sub>2</sub>PO<sub>4</sub> 6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)

20 mM imidazole 1.36 g imidazole (MW 68.08 g/mol)

0.05% Tween 20 5 ml of a 10% Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

### Buffer NPI-250-T (1 liter)

50 mM NaH<sub>2</sub>PO<sub>4</sub> 6.90 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

300 mM NaCl 17.54 g NaCl (MW 58.44 g/mol)

250 mM imidazole 17.00 g imidazole (MW 68.08 g/mol)

0.05% Tween 20 5 ml of a 10% Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

### Buffer B-T (1 liter)

8M Urea 480.50 g urea (MW 60.06 g/mol)

100 mM NaH<sub>2</sub>PO<sub>4</sub> 13.80 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O (MW 137.99 g/mol)

10 mM Tris·Cl 12.10 g Tris·Cl (MW 121.10 g/mol)

0.05% (v/v) Tween 20 5 ml of a 10% (v/v) Tween 20 stock solution

Adjust pH to 8.0 using HCl.

**Buffer C-T (1 liter)**

8M Urea	480.50 g urea (MW 60.06 g/mol)
100 mM NaH <sub>2</sub> PO <sub>4</sub>	13.80 g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (MW 137.99 g/mol)
10 mM Tris·Cl	12.10 g Tris·Cl (MW 121.10 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution
Adjust pH to 6.3 using HCl.	

**Buffer E-T (1 liter)**

8M Urea	480.50 g urea (MW 60.06 g/mol)
100 mM NaH <sub>2</sub> PO <sub>4</sub>	13.80 g NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (MW 137.99 g/mol)
10 mM Tris·Cl	12.10 g Tris·Cl (MW 121.10 g/mol)
0.05% (v/v) Tween 20	5 ml of a 10% (v/v) Tween 20 stock solution
Adjust pH to 4.5 using HCl.	

## Appendix C: Regeneration of *Strep-Tactin Superflow Plus*

*Strep-Tactin Superflow Plus* can be regenerated according to the following procedure.

- 1. Wash the column three times with 5 column volumes *Strep-Tactin Regeneration Buffer*.**

*Strep-Tactin Regeneration Buffer* contains HABA (4-hydroxyazobenzene-2-carboxylic acid). The color change from white to red indicates that the column has been regenerated by displacement of desthiobiotin.

- 2. Wash the column twice with 4 column volumes of *Buffer NP*.**

- 3. Store *Strep-Tactin Superflow* resin in *Buffer NP* at 4°C.**

### Alternatively

- 1. Wash the column with 3 column volumes distilled water using a flow rate of 1 ml/min (1 ml cartridges) or 5 ml/min (5 ml cartridges).**

- 2. Wash the column with 3 column volumes 0.5M NaOH using a flow rate of 0.5–1 ml/min (1 ml cartridges) or 2.5–5 ml/min (5 ml cartridges).**

- 3. Wash the column with 3 column volumes distilled water using a flow rate of 1 ml/min (1 ml cartridges) or 5 ml/min (5 ml cartridges).**

- 4. Re-equilibrate the column with 5 column volumes of *Buffer NP* and store at 4°C.**

## Ordering Information

Product	Contents	Cat. no.
<i>Strep</i> -Tactin Superflow Plus Cartridge (1 ml)	For FPLC purification of <i>Strep</i> -tagged proteins	30020
<i>Strep</i> -Tactin Superflow Plus Cartridge (5 ml)	For FPLC purification of <i>Strep</i> -tagged proteins	30060
<i>Strep</i> -Tactin Superflow Plus (2 ml)	For batch and FPLC purification of <i>Strep</i> -tagged proteins: 2 ml <i>Strep</i> -Tactin-charged resin (max. pressure 140 psi)	30002
<i>Strep</i> -Tactin Superflow Plus (10 ml)	For batch and FPLC purification of <i>Strep</i> -tagged proteins: 10 ml <i>Strep</i> -Tactin-charged resin (max. pressure 140 psi)	30004
<i>Strep</i> -Tactin Magnetic Beads (2 x 1 ml)	For micro-scale purification of <i>Strep</i> -tagged proteins: 2 x 1 ml <i>Strep</i> -Tactin-charged magnetic agarose beads (10% suspension)	36311
<i>Strep</i> -tag Antibody (100 µg)	Mouse monoclonal antibody that recognizes the <i>Strep</i> -tag II epitope; lyophilized, for 1000 ml working solution	34850
<b>Related products</b>		
EasyXpress® Linear Template Kit Plus (20)	For generation of expression constructs using 2-step PCR, 20 reactions	32723
EasyXpress Protein Synthesis Kit (20)*	For 20 x 50 µl in vitro synthesis reactions using <i>E. coli</i> lysates	32502
Ni-NTA Superflow Cartridges (5 x 1 ml)*	5 cartridges pre-filled with 1 ml Ni-NTA Superflow: For FPLC purification of His-tagged proteins	30721
Ni-NTA Superflow (25 ml)*	For batch and FPLC purification of 6xHis-tagged proteins: 25 ml nickel-charged resin (max. pressure 140 psi)	30410

\* Other kit sizes available; see [www.qiagen.com](http://www.qiagen.com).

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
Ni-NTA Magnetic Beads (2 x 1 ml)*	For micro-scale purification of 6xHis-tagged proteins: 2 x 1 ml nickel-charged magnetic agarose beads (5% suspension)	36111
Penta-His Antibody, BSA-free (100 µg)*	100 µg mouse anti-(H) <sub>5</sub> , lyophilized, (BSA-free)	34660

\* Other kit sizes available; see [www.qiagen.com](http://www.qiagen.com).

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

## Notes

## Notes

## Notes

Trademarks: QIAGEN®, QIAexpress®, EasyXpress® (QIAGEN Group); ÅKTAdesign™, ECL™, FPLC™ (Amersham Biosciences); Benzonase® (Merck KGaA, Germany); CDP-Star™ (Applera Corporation or its subsidiaries); Coomassie® (ICI [Imperial Chemical Industries] Organics Inc.); Igepal® (Rhone-Poulenc, Inc.); Strep-tag® and Strep-Tactin® (IBA GmbH); Triton® (Union Carbide Corporation); Tween® (ICI Americas Inc.).

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

Strep-tag technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066 and Strep-Tactin is covered by US patent 6,103,493.

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

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

#### **Limited License Agreement**

Use of this product signifies the agreement of any purchaser or user of the products mentioned in this handbook to the following terms:

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

For updated license terms, see [www.qiagen.com](http://www.qiagen.com).

© 2009–2011 QIAGEN, all rights reserved.

[www.qiagen.com](http://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)

