July 2003

Two-Step Affinity Purification System Handbook

His·*Strep* pQE-TriSystem Vector Set
pQE-TriSystem *Strep* Vector
Strep-Tactin® Superflow
Strep-Tactin Magnetic Beads
Strep-tag® Antibody

For expressing, purifying, and detecting proteins carrying a 6xHis and *Strep*-tag II

QIAGEN

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The *Strep*-Tactin Superflow resin and *Strep*-Tactin protein contained in this product is manufactured by IBA and is provided for use in research and the commercial market. Use in the commercial market is restricted to companies that own a license for the commercial use of the *Strep*-tag II peptide. Information about licenses for commercial use of the *Strep*-tag II peptide is available from IBA GmbH, Rudolf-Wissell-Str. 28, D-37079 Göttingen.

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 (Patent series: RAN 4100/63: USP 4.877.830, USP 5.047.513, EP 253 303 B1), and to 6xHis-coding vectors and His-labeled proteins (Patent series: USP 5.284.933, USP 5.130.663, EP 282 042 B1). All purification of recombinant proteins by Ni-NTA chromatography for commercial purposes, and the commercial use of proteins so purified, require a license from Hoffmann-La Roche.

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Contents

Kit Contents

***Strep*-Tactin® Superflow**		
Catalog no.	**30001**	**30003**
Strep-Tactin Superflow	2 ml	10 ml
Handbook	1	1
***Strep*-Tactin Magnetic Beads**		
Catalog no.	**36311**	**36315**
Strep-Tactin Magnetic Beads	2 x 1 ml	20 x 1 ml
Handbook	1	1
***Strep*-tag Antibody**		
Catalog no.		**34850**
Mouse monoclonal antibody that recognizes the *Strep*-tag II epitope; lyophilized, for 1000 ml working solution		100 µg
Product sheet		1
Handbook		1
His·*Strep* pQE-TriSystem Vector Set		
Catalog no.		**32942**
pQE-TriSystem His·*Strep* 1 and pQE-TriSystem His·*Strep* 2 vectors		25 µg each
Handbook		1
pQE-TriSystem *Strep* Vector		
Catalog no.		**33913**
pQE-TriSystem *Strep* vector		25 µg
Handbook		1

Storage and Stability

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 Two-Step Affinity Purification System is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

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 Two-Step Affinity Purification System or QIAGEN products in general, please do not hesitate to contact us.

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

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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 Two-Step Affinity Purification System:

***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: +49-6131-1924

* 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

Many researchers in structural and functional proteomics use eukaryotic systems, such as insect or mammalian cells, to obtain protein posttranslational 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.

Principle and procedure

Recombinant proteins that carry two small affinity tags (the 6xHis tag and *Strep*-Tag II) are efficiently expressed in *E. coli*, insect, or mammalian cells using pQE-TriSystem His·*Strep* vectors (Figure 1). 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 8). 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 2). The order of purifications can be reversed (i.e., *Strep*-Tactin followed by Ni-NTA purification).

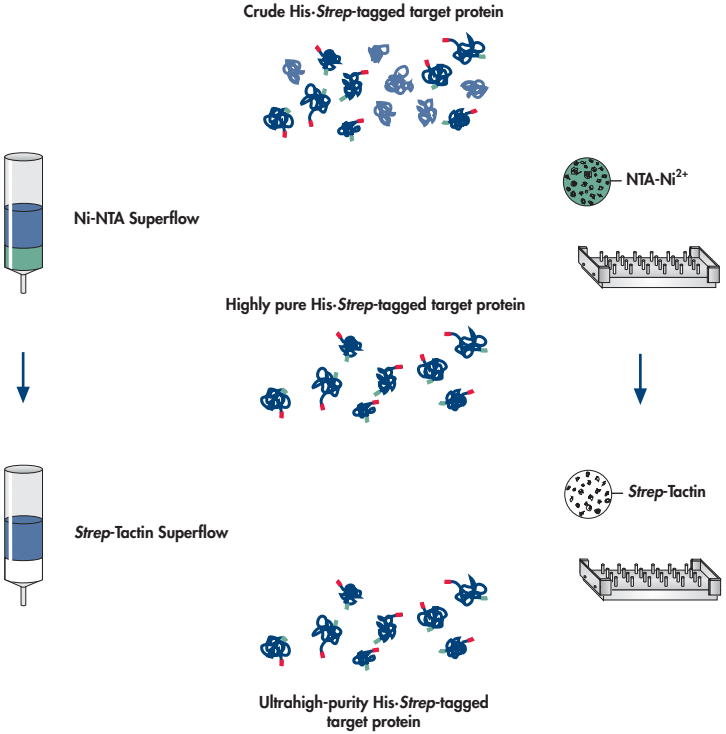
For some applications in which the use of Ni-NTA is problematic, such as purification of metal-binding proteins, reticulocyte lysates, or special buffer conditions (e.g., EDTA), *Strep*-tagged proteins can be purified in a single step using a *Strep*-Tactin matrix.

Proteins containing the *Strep*-Tag II epitope can be detected with high specificity and sensitivity using *Strep*-tag Antibodies (Figure 3).

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.

Two-Step Affinity Purification Procedure



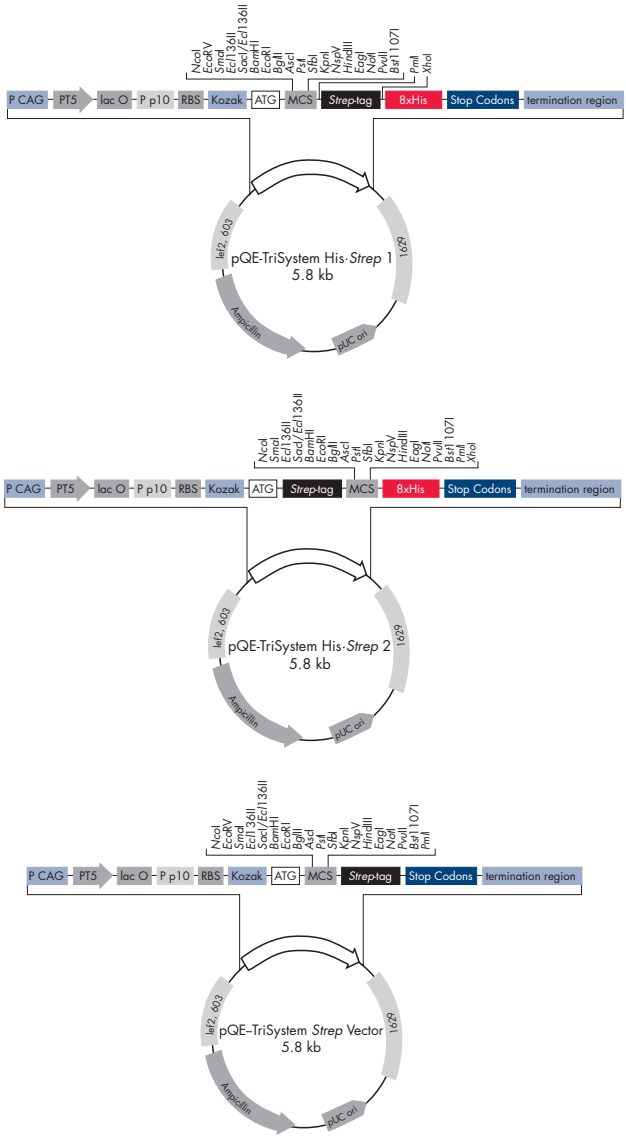


Figure 1 pQE-TriSystem His·*Strep* vectors for parallel protein expression using a single construct in *E. coli*, insect, and mammlian cells. **PT5**: T5 promoter, **lac O**: lac operator, **RBS**: ribosome binding site, **ATG**: start codon, **8xHis**: His tag sequence, **MCS**: multiple cloning site, ***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.

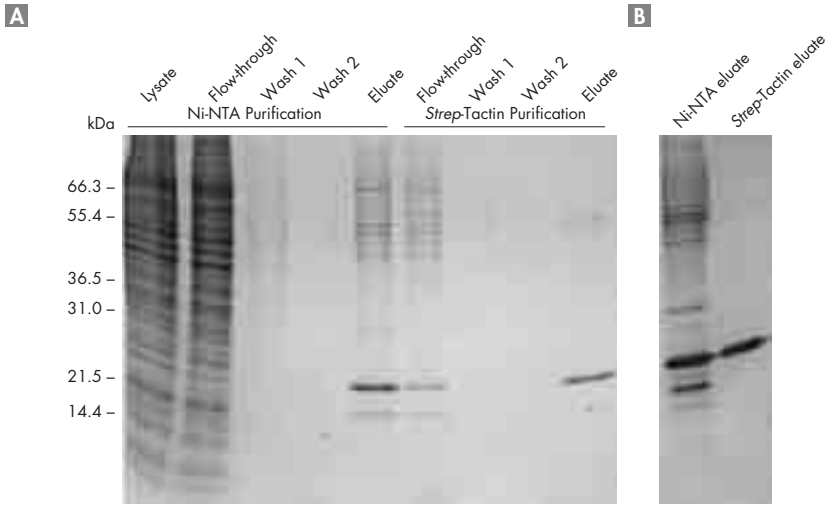


Figure 2 Thioredoxin, expressed in NIH-3T3 cells, was purified using the Two-Step Affinity Purification System. **A** Coomassie®-stained gel. **B** Silver-stained gel.

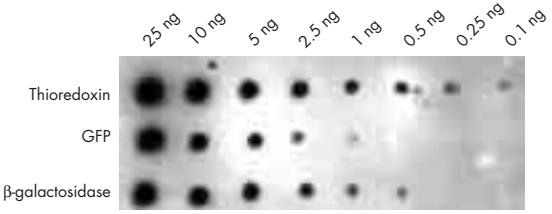


Figure 3 Dot blot showing the sensitivity of *Strep*-tag Antibody. The indicated amounts of protein were spotted onto a membrane and detected using *Strep*-tag Antibody, an anti-mouse secondary antibody conjugated to horseradish peroxidase, and the ECL™ chemiluminescent detection system.

Additional purification using the *Strep*-tag–*Strep*-Tactin interaction

After elution of a His·*Strep*-tagged protein from a Ni-NTA matrix, the *Strep*-tag II allows affinity chromatography on immobilized *Strep*-Tactin under physiological conditions. The *Strep*-tag II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to *Strep*-Tactin, an engineered streptavidin. The binding affinity of the *Strep*-tag II to *Strep*-Tactin (K_d = 1 µM) 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.

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 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. His·*Strep*-tagged proteins expressed in mammalian cells should be purified using Ni-NTA Magnetic Agarose Beads and/or *Strep*-Tactin Magnetic Beads. Table 1 lists the protein binding capacities of Ni-NTA and *Strep*-Tactin matrices.

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

Matrix	Protein binding capacity
Ni-NTA Superflow and Agarose	5–10 mg/ml (250–500 nmol @ ~20 kDa)
Strep-Tactin Superflow	~1 mg/ml (~50 nmol @ ~20 kDa)
Ni-NTA Magnetic Agarose Beads	300 µg/ml (5% suspension) (~15 nmol @ ~20 kDa)
Strep-Tactin Magnetic Beads	200–300 µg/ml (10% suspension) (~10–15 nmol @ ~20 kDa)

Important Notes

Some reagents and buffer components used for protein purification may interfere with the affinity interaction between the protein and purification matrix. Tables 2 and 3 list compatibility of various reagents with the Ni-NTA–6xHis and *Strep*-tag–*Strep*-Tactin interactions.

Table 2. Reagents compatible with the Ni-NTA–6xHis interaction*

6 M guanidine HCl	50% glycerol
8 M urea	20% ethanol
2% Triton® X-100	2 M NaCl
2% Tween® 20	4 M $MgCl_2$
1% CHAPS	5 mM $CaCl_2$
20 mM β-mercaptoethanol	≤20 mM imidazole

Table 3. Reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction*

250 mM imidazole	5 mM DTT
1% Triton X-100	1 M NaCl
1% Tween	10% glycerol
0.3% CHAPS	1 mM EDTA
2% Igepal CA-630 (Nonidet P40)	

* The reagents listed have been successfully used in concentrations up to those given.

Protocol: Growth of *E. coli* Cultures Expressing His·*Strep*-tagged Proteins (50 ml)

Buffer compositions are provided in Appendix A on page 39.

1. **Inoculate 10 ml of LB medium containing ampicillin (100 µg/ml) and kanamycin (25 µg/ml) in a 50 ml flask. Grow the cultures at 37°C overnight.**
2. **Inoculate 50 ml of prewarmed media (with ampicillin) with 2.5 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.**

Protocol: Preparation of Cleared Lysates from *E. coli* Cell Cultures

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

Note: If desired, a single-step purification can be carried out using either *Strep*-Tactin or Ni-NTA Superflow alone. In such cases, use either *Strep*-Tactin Superflow Lysis Buffer or Ni-NTA Lysis Buffer for cell lysis.

The amount of cells required depends on the expression level of the His·*Strep*-tagged protein. For proteins that are expressed at high levels, (10–50 mg of 6xHis-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 His·*Strep*-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 His·*Strep*-tagged protein).

Materials

- 50 or 200 ml culture cell pellet (see note above)
- Lysis Buffer (see note above)
- Lysozyme

Buffer compositions are provided in Appendix A on page 39.

Procedure

1. **Thaw the cell pellet for 15 min on ice and resuspend the cells in 5 ml lysis buffer.**
2. **Add lysozyme to 1 mg/ml and incubate on ice for 30 min.**
3. **Sonicate on ice.**

   Use six 10 s bursts at 200–300 W with a 10 s cooling period between each burst. Use a sonicator equipped with a microtip.
4. **(Optional) If the lysate is very viscous, add RNase A (10 µg/ml) and DNase I (5 µg/ml) and incubate on ice for 10–15 min.**

   Alternatively, draw the lysate through a blunt-ended, narrow-gauge syringe needle several times.
5. **Centrifuge lysate at 10,000 x *g* for 20–30 min at 4°C to pellet the cellular debris. Save supernatant.**

   A certain proportion of the cellular protein may remain insoluble and will be located in the pellet.

6. **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.**

7. **Proceed using one of the purification protocols.**

   See Table 4 to find the protocol best suited to your preparation scale.

Table 4. Protocols for purification of His-*Strep*-tagged proteins from *E. coli* cell lysates

	Expected amount of protein		
	≤50 µg	**>50 µg**	**>>50 µg**
Two-step purification	Magnetic bead protocols, pages 26–29	Superflow batch protocols, pages 20–21	FPLC protocols, pages 22–25
Single-step purification			
Ni-NTA	Magnetic bead protocol, page 26	Superflow batch protocol, page 20	FPLC protocol, page 22
Strep-Tactin	Magnetic bead protocol, page 28	Superflow batch protocol, page 21	FPLC protocol, page 24

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 µg – 10 mg per 10^7 cells.

Note: If the expected yield of the preparation is less than 50 µg, it is highly recommended that proteins be purified using the protocols for Ni-NTA Magnetic Agarose Beads (page 26) and/or *Strep*-Tactin Magnetic Beads (page 28). 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.

If desired, a single-step purification can be carried out using a Ni-NTA or *Strep*-Tactin matrix alone. In such cases, use the lysis buffer applicable to the relevant Ni-NTA or *Strep*-Tactin matrix for cell lysis.

Materials

- Cell pellet
- PBS
- Lysis buffer supplemented with 1% Igepal® CA-630

Buffer compositions are provided in Appendix A on page 39.

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 10^7 cells.**
3. **Centrifuge the lysate at 10,000 x *g* for 10 min at 4°C to pellet cellular debris and DNA. Save the cleared lysate (supernatant).**

   The supernatant should contain the His·*Strep*-tagged protein.
4. **Proceed using one of the purification protocols (see note above).**

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.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Insect-cell growth medium containing secreted proteins
- 10x Binding Buffer (500 mM NaH_2PO_4; 1.5 M NaCl, pH 8.0)

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: Preparation of Lysates from Transfected Mammalian Cells

Recombinant proteins are often expressed in mammalian cells to allow eukaryotic posttranslational 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 µg 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 µg per 10^7 cells. We therefore strongly recommend that proteins expressed in mammalian cells be purified using Ni-NTA Magnetic Agarose Beads and/or *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.

Note: If desired, a single-step purification can be carried out using Ni-NTA or *Strep*-Tactin matrix alone. In such cases, use the lysis buffer applicable to the relevant Ni-NTA or *Strep*-Tactin matrix for cell lysis.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Cell pellet
- PBS
- Lysis buffer for Ni-NTA Magnetic Agarose Beads or *Strep*-Tactin Magnetic Beads (see note above)

Procedure

1. **Wash the transfected cells with phosphate-buffered saline (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 µl lysis buffer per 10^7 cells.**

   If higher concentrations of non-ionic 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 3 on page 12)

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% non-ionic 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. **Centrifuge the lysate at 10,000 x *g* for 10 min at 4°C to pellet cellular debris and DNA. Save the supernatant.**

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

5. **Proceed to protocol for purification using Ni-NTA Magnetic Agarose Beads (page 26) or *Strep*-Tactin Magnetic Beads (page 28).**

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 0.1% Tween, 5–10 mM β-mercaptoethanol, or 1 mM PMSF, or increasing NaCl or glycerol concentrations. See Table 2 on page 12 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Cleared cell lysate
- Ni-NTA matrix
- Empty columns
- Ni-NTA Resin Wash Buffer
- Ni-NTA Resin Elution Buffer

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 Ni-NTA Resin Wash Buffer.**

   Collect wash fractions for SDS-PAGE analysis.
5. **Elute the protein four times with 0.5 ml Ni-NTA Resin Elution Buffer. Collect the eluate in four tubes and analyze by SDS-PAGE.**

Protocol: Batch Purification of His·*Strep*-tagged Proteins Using *Strep*-Tactin Superflow

If desired, His·*Strep*-tagged proteins can be purified directly from cleared *E. coli* lysates using *Strep*-Tactin Superflow. In such cases, *Strep*-Tactin Superflow Lysis Buffer should be used for lysis.

The composition of the lysis, wash and elution buffers can be modified to suit the particular application, e.g., by adding 0.1% Tween, 5 mM DTT, or 1 mM PMSF, or increasing NaCl or glycerol concentrations. See Table 3 on page 12 for a list of buffer reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction.

Buffer compositions are provided in Appendix A on page 39

Materials

- Pooled Ni-NTA column eluates/cleared lysate
- Empty columns
- *Strep*-Tactin Superflow
- *Strep*-Tactin Superflow Lysis Buffer
- *Strep*-Tactin Superflow Elution Buffer

Procedure

1. **Pipet pooled Ni-NTA column eluates or cleared cell lysate into a 15 ml tube and add 2 ml *Strep*-Tactin Superflow 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 *Strep*-Tactin Lysis Buffer.**

   Collect wash fractions for SDS-PAGE analysis.
5. **Elute the protein six times with 0.5 ml *Strep*-Tactin Superflow Elution Buffer. Collect eluates in six tubes and analyze by SDS-PAGE.**

   Strep-Tactin Superflow can be regenerated using the procedure provided in Appendix B on page 41.

Protocol: FPLC Purification of His·*Strep*-tagged Proteins Using Ni-NTA Superflow

The composition of the lysis, wash, and elution buffers can be modified to suit the particular application, for example by adding 0.1% Tween, 5–10 mM β-mercaptoethanol, or 1 mM PMSF, or increasing NaCl or glycerol concentrations. See Table 2 on page 12 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Cleared lysate
- Ni-NTA Superflow
- Empty FPLC column
- Ni-NTA Resin Lysis Buffer
- Ni-NTA Resin Wash Buffer
- Ni-NTA Resin Elution Buffer

Procedure

1. **Assemble the column according to the manufacturer's instructions. Remove the top adapter of the column and cap the bottom outlet.**
2. **Completely resuspend a 50% Ni-NTA Superflow slurry and pour into the column.**

   Avoid introducing air bubbles. Slowly pour the slurry down a thin glass rod inserted into the empty column. The column and bed size depends on the amount of His·*Strep*-tagged protein to be purified. Generally, the binding capacity of Ni-NTA Superflow is 5–10 mg protein per ml resin. Ni-NTA Superflow is supplied as a 50% slurry.
3. **Allow the resin to settle.**

   Allowing the buffer to flow through by uncapping the bottom outlet can accelerate the packing procedure. If desired, a peristaltic pump may be used, but do not exceed flow rates of 2 ml/min. Do not allow resin to dry. If this should occur, resuspend resin in Ni-NTA Resin Lysis Buffer and repack the column. Before the bed has settled, more slurry may be added to increase bed volume.
4. **Insert top adapter and adjust to top of bed.**

   Do not trap any air bubbles. The column can now be connected to the system.
5. **Equilibrate column with 5 column volumes of Ni-NTA Resin Lysis Buffer.**

   The flow rate should not exceed 2 ml/min. Monitor elution at 280 nm; the baseline should be stable after washing with 5 column volumes.

6. **Apply lysate to column and wash with Ni-NTA Resin Lysis Buffer until the A_{280} is stable.**

   Usually 5–10 column volumes are sufficient. Monitor pressure at this step. If the lysate is very viscous, the pressure may exceed the recommended value (10 bar). Reduce flow rate accordingly. Start with a flow rate of 0.5–1 ml/min. If the His·*Strep*-tagged protein does not bind, the flow rate should be reduced. The flow rate may however be increased for protein elution. Collect the flow-through for SDS-PAGE analysis.

7. **Wash with Ni-NTA Resin Wash Buffer until the A_{280} is stable.**

   Usually 5–10 column volumes are sufficient. Collect fractions for SDS-PAGE analysis.

8. **Elute the protein with Ni-NTA Resin Elution Buffer.**

   If desired, a step-gradient of elution buffer in wash buffer may be used to elute the protein. Five column volumes at each step are usually sufficient. The His·*Strep*-tagged protein is usually eluted in the second and third column volumes.

   Note: Imidazole absorbs at 280 nm, which should be considered when monitoring protein elution. If small amounts of His·*Strep*-tagged proteins are purified, elution peaks may be poorly visible.

Protocol: FPLC Purification of His·*Strep*-tagged Proteins Using *Strep*-Tactin Superflow

The composition of the lysis and elution buffers can be modified to suit the particular application, for example by adding 0.1% Tween, 5 mM DTT, or 1 mM PMSF, or increasing NaCl or glycerol concentrations. See Table 3 on page 12 for a list of buffer reagents compatible with the *Strep*-tag–*Strep*-Tactin interaction.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Cleared lysate
- Empty columns
- *Strep*-Tactin Superflow
- *Strep*-Tactin Superflow Lysis Buffer
- *Strep*-Tactin Superflow Elution Buffer

Procedure

1. **Assemble the column according to the manufacturer's instructions. Remove the top adapter of the column and cap the bottom outlet.**
2. **Completely resuspend a *Strep*-Tactin Superflow slurry and pour into the column.**

   Avoid introducing air bubbles. Slowly pour the slurry down a thin glass rod inserted into the empty column. The column and bed size depends on the amount of His·*Strep*-tagged protein to be purified. Generally, the binding capacity of *Strep*-Tactin Superflow is 1 mg protein per ml resin. *Strep*-Tactin Superflow is supplied as a 50% slurry.
3. **Allow the resin to settle.**

   Allowing the buffer to flow through by uncapping the bottom outlet can accelerate the packing procedure. If desired, a peristaltic pump may be used, but do not exceed flow rates of 2 ml/min. Do not allow resin to dry. If this should occur, resuspend resin in *Strep*-Tactin Superflow Lysis Buffer and repack the column. Before the bed has settled, more slurry may be added to increase bed volume.
4. **Insert top adapter and adjust to top of bed.**

   Do not trap any air bubbles. The column can now be connected to the system.
5. **Equilibrate column with 5 column volumes of *Strep*-Tactin Superflow Lysis Buffer.**

   The flow rate should not exceed 2 ml/min. Monitor elution at 280 nm; the baseline should be stable after washing with 5 column volumes.

6. **Apply lysate to column and wash with *Strep*-Tactin Superflow Lysis Buffer until the A_{280} is stable.**

   Usually 8–10 column volumes are sufficient. Monitor pressure at this step. If the lysate is very viscous, the pressure may exceed the recommended value (10 bar). Reduce flow rate accordingly. Start with a flow rate of 0.5–1 ml/min. If the His·*Strep*-tagged protein does not bind, the flow rate should be reduced. The flow rate may however be increased for protein elution. Collect the flow-through for SDS-PAGE analysis.

7. **Elute the protein with *Strep*-Tactin Superflow Elution Buffer.**

   Five column volumes at each step are usually sufficient. The His·*Strep*-tagged protein is usually eluted in the third and fourth column volumes. *Strep*-Tactin Superflow can be regenerated using the procedure provided in Appendix B on page 41.

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 0.1% Tween, 5–10 mM β-mercaptoethanol, or 1 mM PMSF, or increasing NaCl or glycerol concentrations. See Table 2 on page 12 for a list of buffer reagents compatible with the 6xHis-tag–Ni-NTA interaction.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Cleared lysate
- Ni-NTA Magnetic Agarose Beads
- Ni-NTA Beads Lysis Buffer
- Ni-NTA Beads Wash Buffer
- Ni-NTA Beads Elution Buffer

Procedure

1. **Resuspend the Ni-NTA Magnetic Agarose Beads by vortexing for 2 s and then immediately add 200 µl 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 µl magnetic-bead suspension has a binding capacity of 60 µg 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 µl 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 dilute lysates which have been concentrated 5-fold, and if using native lysis conditions, add RNase A to 10 µg/ml and DNase I to 5 µg/ml, 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 Ni-NTA Beads Wash Buffer, 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 Ni-NTA Beads Elution Buffer, 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.

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

If desired, His·*Strep*-tagged proteins can be purified directly from cleared *E. coli*-, insect-, or mammalian-cell lysates using *Strep*-Tactin Magnetic Beads. In such cases, *Strep*-Tactin Beads Lysis Buffer should be used for lysis.

The composition of the lysis and elution buffers can be modified to suit the particular application, for example by adding 0.1% Tween, 5 mM DTT, or 1 mM PMSF, or increasing NaCl or glycerol concentrations. See Table 3 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.

Buffer compositions are provided in Appendix A on page 39.

Materials

- Cleared lysate or eluate from Ni-NTA Magnetic Beads
- *Strep*-Tactin Magnetic Beads
- Ni-NTA Magnetic Agarose Beads
- *Strep*-Tactin Beads Elution Buffer

Procedure

1. **Resuspend *Strep*-Tactin Magnetic Beads by vortexing for 2 s and then immediately add 200 µl 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 µl of *Strep*-Tactin Magnetic Beads suspension has a binding capacity of 40–60 µg 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 µl 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 RNase A to 10 µg/ml and DNase I to 5 µg/ml, 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 µl *Strep*-Tactin Beads Lysis Buffer, 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 µl *Strep*-Tactin Beads Elution Buffer, 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.

For compositions and preparation of buffers and reagents, see Appendix A, page 39.

Materials

- 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 PBS)
- *Strep*-tag Antibody stock solution: Dissolve the lyophilized *Strep*-tag Antibody in 500 µl water. The reconstituted solution contains 200 µg/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)

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 5.

Table 5. Reagents used in chemiluminescent detection of *Strep*-tagged proteins

Step		(Alternative method)
Blocking	3% BSA in TBS	1% Casein in TBS
Strep-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™ from Tropix, Inc. can be used with AP-conjugated secondary antibodies, and the ECL system from Amersham Pharmacia Biotech 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.**
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.**
4. **Wash membrane for 10 min with TBS buffer at room temperature.**
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 this blocking step when using chemiluminescent detection.
6. **Wash twice for 10 min each time in TBS-Tween/Triton buffer at room temperature.**
7. **Wash for 10 min in TBS buffer at room temperature.**
8. **Incubate with secondary antibody solution for 1 h at room temperature.**

   Either alkaline phosphatase (AP) or horseradish peroxidase (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.**
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.

Materials

- 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 alkaline phosphatase (AP) or horseradish peroxidase (HRP) conjugate
- Blocking buffer (3% BSA in TBS)
- Secondary antibody dilution buffer (3% BSA in TBS)
- Staining solutions for alkaline phosphatase (AP) or horseradish peroxidase (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.**
2. **Incubate for 1 h in blocking buffer at room temperature.**

   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.**

   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.**
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.**

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.**

   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. Dilute according to the manufacturer's recommendations. Use the lowest recommended amounts to avoid false signals.
9. **Wash 4 times for 10 min each time in TBS-Tween/Triton buffer at room temperature.**

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.**

    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. 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 molecular biology applications (see inside front cover for contact information).

Purification using Ni-NTA matrices

	Comments and suggestions
Protein does not bind to the Ni-NTA resin	
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).
6xHis tag is inaccessible	Move tag to the opposite end of the protein.
6xHis tag has been degraded	Check that the 6xHis tag is not associated with a portion of the protein that is processed.
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.
Protein elutes in the wash buffer	Wash stringency is too high. Lower the concentration of imidazole or increase the pH slightly.
6xHis tag is partially hidden	Reduce wash stringency.
Buffer conditions incorrect	Check pH and composition of wash buffer. Ensure that there are no chelating or reducing agents present.
Protein precipitates during purification	Temperature is too low. Perform purification at room temperature.
Protein forms aggregates	Try adding solubilization reagents such as 0.1% Triton X-100 or Tween-20, up to 20 mM β-ME, up to 2 M NaCl, or stabilizing cofactors such as Mg^{2+}. These may be necessary in all buffers to maintain protein solubility
Protein does not elute	
Elution conditions are too mild (protein may be in an aggregate or multimer form)	Elute with a pH or imidazole step gradient to determine the optimal elution conditions.

	Comments and suggestions
Protein has precipitated in the column	Perform binding and elution in batch format to avoid high local protein concentrations.
Protein does not elute	
Binding and wash conditions not stringent enough	Include 10–20 mM imidazole in the binding and wash buffers.
Contaminants are associated with tagged protein	Add β-ME to a maximum of 20 mM to reduce disulfide bonds. Increase salt and/or detergent concentrations, or add ethanol/glycerol to wash buffer to disrupt nonspecific interactions.
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.
Discoloration of resin	
Nickel ions are removed or reduced	Ensure that there are no chelating compounds (resin turns white in color) or reducing agents (resin turns brown in color) present in the buffers.
Purification from mammalian cells	
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 *QIA*express *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.
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.

	Comments and suggestions
6xHis-tagged protein partially elutes in the wash	The binding capacity used is too low to bind all of the 6xHis-tagged protein. 10 µl magnetic-bead suspension has a binding capacity of 3 µg 6xHis-tagged DHFR (24 kDa). If significantly larger amounts of 6xHis-tagged protein are present in the lysate, increase the amount of beads accordingly.
Binding of contaminants	
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.
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.
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	
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 *QIA*express *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
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. Work at 4°C and add protease inhibitors, such as PMSF.
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 µl Ni-NTA agarose has a binding capacity of 500–1000 µg 6xHis-tagged protein. Adjust the amount of matrix used for purification accordingly.
Contaminants bind to resin	
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.
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.
Purification using *Strep*-Tactin matrices	
Protein does not bind to the *Strep*-Tactin matrix	
Binding conditions are the 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 *Strep*-tag II–*Strep*-Tactin interaction. Check buffer pH, which should be above 6.5.
Strep-Tactin matrix binding sites are occupied/blocked	Biotin irreversibly blocks the binding sites of *Strep*-Tactin 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 *Strep*-tag II–*Strep*-Tactin interaction.

	Comments and suggestions
Strep-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).
Strep-tag is inaccessible	Move tag to the opposite end of the protein.
Strep-tag–*Strep*-Tactin interaction is very weak	Replace batch purification by applying the lysate to a prepared column. Use more concentrated lysate or Ni-NTA eluate and remove *Strep*-Tactin Magnetic Beads storage buffer prior to use.
Protein does not elute from *Strep*-Tactin resin	The *Strep*-tag II–*Strep*-Tactin interaction has a K_D value in the µM 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.
Protein has precipitated on the column	Add detergents to or reduce salt concentration in buffers. Do not use a pH near the pI of the protein. Increase or lower pH (pH must however, be above 6.5).

Appendix A: Composition of Buffers

LB medium

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

2x SDS-PAGE sample buffer

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

5x SDS-PAGE sample buffer

0.225 M Tris·Cl; 50% glycerol; 5% SDS; 0.05% bromophenol blue;
0.25 M DTT, pH 6.8

10x Binding Buffer (1 liter):

500 mM NaH_2PO_4	69.0 g $NaH_2PO_4 \cdot H_2O$ (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 Ni-NTA Superflow

Ni-NTA Superflow Lysis Buffer* (1 liter):

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

Adjust pH to 8.0 using NaOH.

Ni-NTA Superflow Wash Buffer (1 liter):

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

Adjust pH to 8.0 using NaOH.

Ni-NTA Superflow Elution Buffer (1 liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
250 mM imidazole	17.00 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.

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

PBS (1 liter):

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

Adjust pH to 7.2 using NaOH.

Ni-NTA Beads Lysis Buffer (1 liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
10 mM imidazole	0.68 g imidazole (MW 68.08 g/mol)
0.05% Tween 20	5 ml of a 10% Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

Ni-NTA Beads Wash Buffer (1 liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
20 mM imidazole	1.36 g imidazole (MW 68.08 g/mol)
0.05% Tween 20	5 ml of a 10% Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

Ni-NTA Beads Elution Buffer (1 liter):

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
250 mM imidazole	17.00 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.

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

***Strep*-Tactin Superflow Lysis Buffer (1 liter):**

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

Adjust pH to 8.0 using NaOH.

***Strep*-Tactin Superflow Elution buffer (1 liter):**

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
2.5 mM desthiobiotin	0.54 g desthiobiotin (Sigma cat. no. D 1411)

Adjust pH to 8.0 using NaOH.

***Strep*-Tactin Superflow Regeneration Buffer (1 liter):**

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
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 *Strep*-Tactin Magnetic Beads

***Strep*-Tactin Beads Lysis Buffer (1 liter):**

50 mM NaH_2PO_4	6.90 g $NaH_2PO_4 \cdot H_2O$ (MW 137.99 g/mol)
300 mM NaCl	17.54 g NaCl (MW 58.44 g/mol)
0.05% Tween 20	5 ml of a 10% Tween 20 stock solution

Adjust pH to 8.0 using NaOH.

***Strep*-Tactin Beads Elution Buffer (1 liter):**

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

Appendix B: Regeneration of *Strep*-Tactin Superflow

Strep-Tactin Superflow can be regenerated according to the following procedure. The resin can be regenerated a maximum of two times and should then be discarded.

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

Ordering Information

Product	Contents	Cat. No.
Strep-Tactin Superflow (2 ml)	For batch and HPLC purification of *Strep*-tagged proteins: 2 ml *Strep*-Tactin–charged resin (max. pressure: 140 psi)	30001
Strep-Tactin Superflow (10 ml)	For batch and HPLC purification of *Strep*-tagged proteins: 10 ml *Strep*-Tactin–charged resin (max. pressure: 140 psi)	30003
Strep-Tactin Magnetic Beads (2 x 1 ml)	For micro-scale purification of *Strep*-tagged proteins: 2 x 1 ml *Strep*-Tactin–charged magnetic agarose beads (10% suspension)	36311
Strep-Tactin Magnetic Beads (20 x 1 ml)	For micro-scale purification of *Strep*-tagged proteins: 20 x 1 ml *Strep*-Tactin–charged magnetic agarose beads (10% suspension)	36315
Strep-tag Antibody (100 µg)	Mouse monoclonal antibody that recognizes the *Strep*-tag II epitope; lyophilized, for 1000 ml working solution	36315
His·*Strep* pQE-TriSystem Vector Set	pQE-TriSystem His·*Strep* 1 and pQE-TriSystem His·*Strep* 2 vectors, 25 µg each	32942
pQE-TriSystem *Strep* Vector	pQE-TriSystem *Strep* vector, 25 µg	33913
Related products		
Ni-NTA Superflow (25 ml)*	For batch and HPLC purification of 6xHis-tagged proteins: 25 ml nickel–charged resin (max. pressure: 140 psi)	30410
Ni-NTA Magnetic Agarose Beads (2 x 1 ml)*	For micro-scale purification of 6xHis-tagged proteins: 2 x 1 ml nickel charged magnetic agarose beads (5% suspensionf	36111
Penta·His Antibody, BSA-free (100 µg)*	100 µg mouse anti-$(H)_5$ (lyophilized, BSA-Free, for 1000 ml working solution)	34660
Tetra·His Antibody, BSA-free (100 µg)*	100 µg mouse anti-$(H)_4$ (lyophilized, BSA-Free, for 1000 ml working solution)	34660

* Larger sizes available; please inquire.

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