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Expression and purification of proteins using Strep-Tactin®XT

A comprehensive manual

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Content

For Twin-Strep-tag® purification from **mammalian cells** a separate manual is available (https://www.iba-lifesciences.com).

1 Introduction

1.1 Strep-tag® technology overview

The Strep-tag technology is a widely used affinity tag based protein purification system. It comprises two tags – the eight amino acid Strep-tag®II and the Twin-Strep-tag® a tandem version of the Strep-tag®. These two tags can be freely combined with one of the two available ligands – Strep-Tactin® and Strep-Tactin®XT. The tag/ligand combination depends on the required binding strength and application. While the Strep-tag®II has an affinity in the µM or nM range for Strep-Tactin® and Strep-Tactin®XT, the Twin-Strep-tag® has a nM affinity for Strep-Tactin® and a pM affinity for Strep-Tactin®XT. This high affinity is not achieved by any other existing affinity tag system. In addition this flexibility in combining the tag and ligand allows the best possible coordination of the purification conditions for recombinant proteins under physiological conditions.

The Strep-tag® technology can be used to purify functional Strep-tagged proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria [1, 2, 3]. Tolerating diverse buffer conditions and additives (high salt, detergents, reducing agents, metal ions and chelating agents), the technology is universally applicable for any protein properties and even convenient for protein complexes and protein interaction analysis. Generally, both tags does not interfere with folding or bioactivity of the target protein, does not react with heavy metal ion buffer impurities, has no ion exchange properties and does not induce protein aggregation. Thus, there is no need for removal of Strep-tag®II and Twin-Strep-tag®.

In addition, the Twin-Strep-tag®/Strep-Tactin®XT combination is ideally suited for immobilization and detection in analytic applications like ELISA or SPR. This over all versatility makes the Strep-tag® technology superior to all other available tag-based affinity purification systems.

1.2 The Strep-tag® technology protein purification principle

The basis for the development of the Strep-tag® technology has been the well-known binding of biotin to streptavidin (Fig. 1). To take advantage of this strong interaction in protein purification applications we put effort in finding a peptide that is capable of binding to the biotin binding pocket of streptavidin when fused to recombinant proteins. This peptide was supposed to serve as purification tag. The finally engineered short sequence consists of only eight amino acids (sequence: WSHPQFEK) and was named Strep-tag®II. To optimize binding properties, also streptavidin has been engineered to obtain Strep-Tactin®. Constant research led to further developments and finally resulted in the 3rd generation of the Strep-tag® technology: Strep-Tactin®XT and Twin-Strep-tag®.

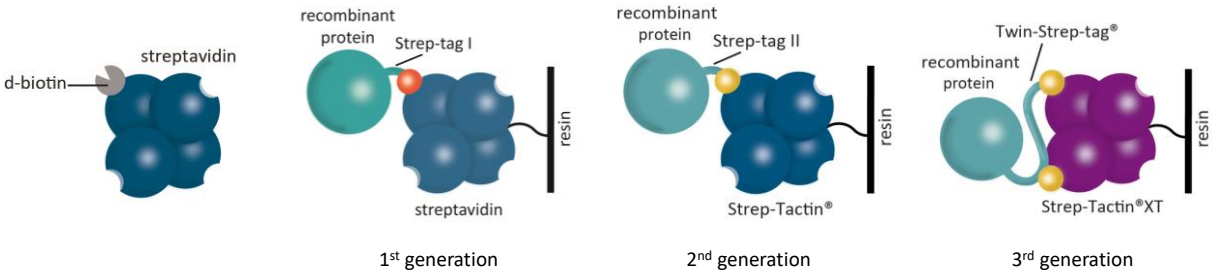


Fig. 1: Schematic view of the Strep-tag® core technology.

Strep-tag®II and Twin-Strep-tag® in comparison

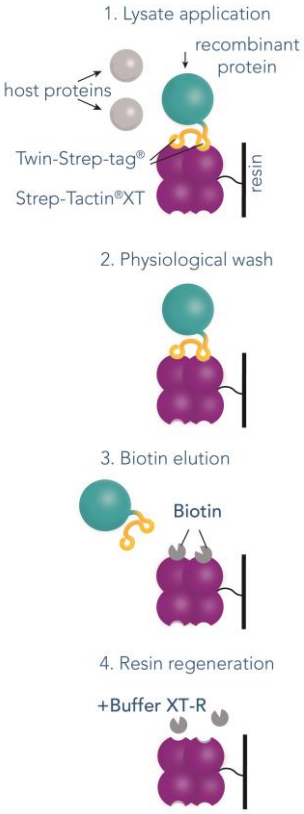
The Strep-tag®II is a short peptide tag with negligible effect on the recombinant protein due to its chemically balanced amino acid composition (8 amino acids, WSHPQFEK) which can be fused to the protein as either N- or C-terminal tag. A two amino acid spacer (SerAla) between the protein and the tag promotes the accessibility of the tag. The further improved Twin-Strep-tag® is a sequential arrangement of two Strep-tag®II sequences with an internal linker region (total size of 28 aa). This tag enables the same mild and rapid purification as Strep-tag®II but, in addition, has an increased affinity for Strep-Tactin® and Strep-Tactin®XT which allows efficient purification even in batch or directly from cell culture supernatants.

Strep-Tactin®XT

Strep-Tactin®XT is the further development of Strep-Tactin®. The interaction between Strep-Tactin®XT and Twin-Strep-tag® has a binding affinity in the low pM enabling the superior performance compared to all other available purification systems. The near covalent binding of the interaction partners ensures higher protein yields compared to Strep-Tactin® and makes the system suitable for purifications under various conditions. Moreover, the neutral pI of Strep-Tactin®XT minimizes non-specific protein or nucleic acid binding.

1.3 The Strep-Tactin®XT protein purification cycle

The purification of Strep-tag®II and Twin-Strep-tag® fusion proteins is easy, straightforward and user-friendly. The complete procedure can be performed under physiological conditions, e.g. in PBS buffer. Physiological buffers can optionally be used in combination with a wide range of additives. Elution is simply induced by addition of biotin (Fig. 2). Biotin is the natural ligand of streptavidin and therefore conduces to for the mild elution of Strep-tagged proteins. Column regeneration is easily achieved by applying Buffer XT-R (3 M $MgCl_2$, Cat. No. 2-1045-025). Alternatively, freshly prepared 10 mM NaOH solution can be used.



Step 1: The cell lysate or culture supernatant is applied on the column.

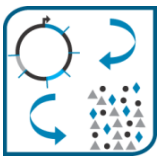
Step 2: Once the tagged protein has bound specifically to Strep-Tactin®XT the host proteins are instantly washed away with moderate amounts of physiological wash buffer (Buffer W).

Step 3: Bound Twin-Strep-tag® protein (shown) or Strep-tag®II protein (not shown) are gently eluted by wash buffer containing 50 mM biotin (Buffer BXT) which specifically competes for the biotin binding pocket.

Step 4: Regeneration of the column is achieved by the application of Buffer XT-R (3 M $MgCl_2$) or alternatively by freshly prepared 10 mM NaOH. Buffer XT-R can be removed simply by applying Buffer W. Strep-Tactin®XT resin can be regenerated and re-used 3 to 5 times without loss in performance.

Fig. 2: Strep-Tactin®XT purification cycle

2 Expression in *E. coli*



Even minute quantities of recombinant protein caused by expression of a leaky promotor can influence *E. coli* cell growth. Moreover, it can result in a dramatic selection against *E. coli* cells harboring the expression plasmid in case the recombinant protein is cytotoxic. Consequently, regulation of heterologous protein biosynthesis is generally recommended by the use of a promoter whose activity can be blocked by a repressor, and subsequent synthesis of the gene product is switched on in a controlled manner by adding a chemical inducer.

IBA offers *E. coli* expression vectors using two different repression and induction systems: vectors either harboring the *tet* promotor (see **2.1**) or the T7 promotor (see **2.2**).

2.1 Expression in *E. coli* with the *tet*-system (pASK-IBA /pASG-IBA vectors)

The Twin-Strep-tag® and Strep-tag®II expression vectors pASK-IBA/pASG-IBA carry the promoter/operator region from the *tet*A resistance gene [4, 5]. The strength of the *tet*A promoter is comparable with that of the *lac*-UV5 promoter.

The protein expression can be induced by adding anhydrotetracycline at a concentration that is antibiotically not effective (200 ng/ml). The constitutive expression of the *tet* repressor gene, which is also encoded on the expression plasmids, guarantees the repression of the promoter in the absence of the inducer. In a Western blot, no expression is detectable under these conditions [4, 6]. In contrast to the *lac* promoter, which is susceptible to catabolite repression (cAMP-level, metabolic state) and dependent on chromosomally encoded repressor molecules, the *tet*A promoter/operator is not coupled to any cellular regulation mechanisms. Thus, there are basically no restrictions in the choice of culture medium or *E. coli* expression strain. For example, glucose minimal media and even the bacterial strain XL1-Blue, which carries an episomal copy of the tetracycline resistance gene, can be used for expression.

IBA offers a special developed Mammalian expression system (MEXi) which consists of HEK-293 cells adapted to suspension growth in chemical defined media with a very low biotin concentration. Corresponding manuals on https://www.iba-lifesciences.com.

Recommended buffers/solutions	Concentration of ingredients	Notes
ampicillin (pASG-IBA vectors, pASK-IBA vectors except: pASK-IBA2C, 3C, 4C, 5C, 6C, 7C)	stock solution 100 mg/ml in H_2O, sterile filtered	Store in aliquots at –20°C.
chloramphenicol (pASK-IBA2C, 3C, 4C, 5C, 6C, 7C)	stock solution 30 mg/ml in ethanol	Recommended for fermentation at high cell densities. Store at –20°C.
anhydrotetracycline	stock solution 2 mg/ml in dimethylformamid (DMF)	Store at –20°C.
LB medium	10 g/l trypton 5 g/l yeast extract 5 g/l NaCl	
Buffer W	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	
5 x SDS-PAGE sample buffer	250 mM Tris/HCl, pH 8.0 25% glycerol 7,5% SDS 0.25 mg/ml bromophenol blue 12.5% v/v 2-mercaptoethanol	

Important notes

The *tet* promoter system is independent of the *E. coli* strain. Following strains were successfully tested: JM83, WK6, B, BL21, MG1655, W3110, XL1-Blue, BL21-CodonPlus™

→ We recommend JM83 or W3110 for periplasmic secretion.

Protocol

1. **Preculture: inoculate 2 ml of LB medium containing 100 µg/ml ampicillin (pASG/pASK-IBA plasmids except 2C to 7C) or 30 µg/ml chloramphenicol (pASK-IBA2C to 7C) with a fresh colony harboring the expression plasmid and shake overnight (200 rpm) at 37°C.**
   Please note:
   - The colony should not be older than 1 week.
   - We recommend using overnight colonies.
   - Do not inoculate from glycerol stocks.

   In most cases, the yield of soluble, functional protein can be significantly increased by lowering the growth temperature of the preculture to 22–30°C. The cells should not reach the stationary phase for extended periods prior to inoculation.

2. **Culture for expression: Inoculate 100 ml of LB medium containing 100 µg/ml ampicillin (or 30 µg/ml chloramphenicol, depending on the plasmid used) with the preculture and shake at 37°C.**

3. **Monitor the optical density at 550 nm (OD_{550}).** Cell suspension samples with an OD_{550} higher than 1.0 should be diluted with LB medium before measuring.

4. **When OD_{550} equals 0.5–0.6, add 10 µl of anhydrotetracycline stock solution.** The yield of soluble, functional protein may be substantially increased, particularly in case of periplasmic expression, by lowering the growth temperature to 22–30°C. **Take a 1 ml sample immediately before induction.** This sample represents the non-induced control; pellet cells (microfuge, 30 seconds) and resuspend them in 80 µl Buffer W. Add 20 µl 5 x SDS-PAGE sample buffer and mix. Store at –20°C until SDS-PAGE analysis.

5. **Shake for 3 hours at 200 rpm.** Overnight expression may increase protein yields in some cases.

6. **Harvest the cells by centrifugation at 4500 x g for 12 minutes (4°C).**

Proceed to 3 (Preparation of cleared lysates) or store cell pellet at –20°C

2.2 Expression in *E. coli* with the T7-system (pPSG-IBA vectors)

The system uses the T7 promoter and T7 RNA polymerase for high-level transcription of the gene of interest. Since the T7 promoter is stronger than the *tet* promoter, pPSG-IBA vectors can be recommended in cases where expression with the *tet* promoter does not lead to sufficient yields of the recombinant protein.

Expression of the target gene is induced by providing a source of T7 RNA polymerase in the *E. coli* host cell (DE3 lysogen). This is accomplished by using e.g. *E. coli* BL21(DE3) host which contains a chromosomal copy of the T7 RNA polymerase gene (Novagen, Invitrogen). The T7 RNA polymerase gene is under control of the lacUV5 promoter which can be induced by IPTG [6, 7].

Recommended buffers/solutions	**Concentration of ingredients**	**Notes**
Ampicillin (pPSG-IBA vectors)	stock solution 100 mg/ml in H_2O, sterile filtered	Store in aliquots at –20°C
LB medium	10 g/l trypton 5 g/l yeast extract 5 g/l NaCl	
IPTG (Isopropyl β-D-1-thiogalactopyranoside)	stock solution (1 M): 238 mg/ml in H_2O, sterile filtered	Store in aliquots at –20°C
Glucose	20%, sterile filtered	
Buffer W	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	
5x SDS-PAGE sample buffer	250 mM Tris/HCl, pH 8.0 25% glycerol 7,5% SDS 0.25 mg/ml bromophenol blue 12.5% v/v 2-mercaptoethanol	

Protocol

1. **Preculture: Inoculate 2 ml of LB medium containing 100 µg/ml ampicillin with a fresh colony harboring the pPSG-IBA expression plasmid and shake overnight (200 rpm) at 37°C.**
   Please note:
   - The colony should not be older than 1 week.
   - We recommend using overnight colonies.
   - Do not inoculate from glycerol stocks.

   The yield of soluble, functional protein can often be significantly increased by lowering the growth temperature of the preculture to 22–30°C. The cells should not reach the stationary phase for extended periods prior to inoculation.

   In case of toxic proteins, the leakiness of the lacUV5 promoter and the resulting expression may lead to cell death or to the selection of non-productive mutants. Add 2% glucose and/or use pLysS or pLysE co-transformants in such cases in order to prevent basal expression [7].

2. **Culture for expression: Inoculate 100 ml of LB medium containing 100 µg/ml ampicillin with the preculture and shake at 37°C.**

3. **Monitor the optical density at 550 nm (OD_{550}).** Cell suspension samples with an OD_{550} higher than 1.0 should be diluted with LB medium before measuring.

4. **When OD_{550} equals 0.5–0.6, add 50 µl of IPTG stock solution (0.5 mM end concentration).** The yield of soluble, functional protein may be substantially increased, particularly in case of periplasmic expression, by lowering the growth temperature to 22–30°C. **Take a 1 ml sample immediately before induction.** This sample represents the non-induced control; pellet cells (microfuge, 30 seconds) and resuspend them in 80 µl Buffer W. Add 20 µl 5 x SDS-PAGE sample buffer and mix. Store at –20°C until SDS-PAGE analysis.

5. **Shake for 3 hours at 200 rpm.** Overnight expression may increase protein yields in some cases.

6. **Harvest the cells by centrifugation at 4500 x g for 12 minutes (4°C).**

Proceed to 3 (Preparation of cleared lysates) or store cell pellet at –20°C.

2.3 Expression with other systems

Bacterial expression has the advantage of obtaining the expression product in a short time at low cost. Nevertheless, there are proteins which cannot be expressed in *E. coli*. In such cases yeast, insect, mammalian or plant cells can be used as alternative expression hosts. IBA´s StarGate Cloning system provides a variety of expression vectors for these hosts (yeast, mammalian and insect cells (pYSG-, pESG-, pCSG- and pLSG-IBA vectors)) for a multitude of affinity tags (Strep-tag®, Twin-Strep-tag®, 6xHistidine-tag, FLAG-tag and GST-tag).

2.4 Precautions to prevent Strep-Tactin®XT blocking through biotin

Free biotin binds to Strep-Tactin®XT. Thereby at high concentrations it reduces the binding capacity of the resin for the protein of interest. Biotin can be removed or masked prior to affinity chromatography. The best and simplest precaution is to add IBA's Biotin blocking solution (BioLock) or stoichiometric amounts of avidin for irreversible masking prior to chromatography. Other solutions are removal via dialysis, ammonium sulfate precipitation or cross-flow filtration/concentration.

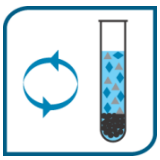
The MEXi system provides mammalian cell culture media with a minimum biotin concentration for effective Strep-Tactin®XT:Twin-Strep-tag® purification. However, the Biotin concentration in most cell culture supernatants (including Expi media) is not critical and supernatant can be loaded directly on the Strep-Tactin®XT resin. The biotin issue is most relevant when cell culture lysate containing biotinylated recombinant protein is directly subjected to Strep-Tactin®XT resins, since biotinylated proteins can be co-purified with the protein of interest.

The amount of biotinylated proteins or free biotin in all extracts is rather low and does not affect binding capacity of Strep-Tactin®XT significantly. Cell internal biotin content in e.g. *E. coli* is about 7 nmol/l/OD. An overview on biotin contents of standard cell culture media can be found at https://www.iba-lifesciences.com.

2.5 Troubleshooting – Expression

Problem	Comments and suggestions
No or low expression	• Check the culture condition (e.g. IPTG, anhydrotetracycline, antibiotics). • Check vector (sequence, frame). • Check whether the protein is found in the insoluble fraction. • Reduction of temperature during cultivation may solve this problem (e.g. 16, 22, 26, or 30°C). • Use another expression system (e.g. T7 promoter instead of *tet* promoter, see page 10). • Use eukaryotic cells for expression (yeast, insect or mammalian cells).
Protein is degraded.	• Use protease deficient *E. coli* strains. • If degradation occurs during cell lysis, add protease inhibitor. • If the protein is small (<10 kDa), consider adding a terminal carrier protein. • Lower temperature during expression can reduce the problem. • Secretion of the recombinant protein to the periplasmic space can reduce the problem.
Protein is secreted.	• Remove all signal sequences from the coding region.
Inclusion bodies are formed – protein is insoluble.	• Reduce expression level by modifying growth and induction conditions, e.g.: lower culturing temperature (16, 22, 26, or 30°C). • Use another expression system (e.g. *tet* promoter instead of T7 promoter, see 2.1).

3 Preparation of cleared lysates



3.1 Preparation of cleared lysate after cytoplasmic expression of Strep-tag®II and Twin-Strep-tag® fusion proteins

Recommended buffers/solutions	Concentration of ingredients	Notes
Buffer W	100 mM Tris/HCl pH 8.0 150 mM NaCl 1 mM EDTA	10 x Buffer W has to be diluted before application. It is recommended to work without EDTA when metalloproteins have been expressed.
5 x SDS-PAGE sample buffer	250 mM Tris/HCl, pH 8.0 25% glycerol 7.5% SDS 0.25 mg/ml bromophenol blue 12.5% v/v 2-mercaptoethanol	

Protocol

1. **Chill Buffer W at 4°C.**

2. **Resuspend pellet of a 100 ml culture in 1 ml Buffer W.**

3. **Take a 10 µl sample for analysis of the total protein content via SDS-PAGE and/or Western blotting.** The 10 µl sample should be thoroughly mixed with 90 µl Buffer W and 25 µl of 5 x SDS-PAGE sample buffer. Store it at –20°C. The whole sample must be incubated in an ultrasonic bath for 15 minutes to shear the chromosomal DNA into small pieces and should be heated to 70°C for 10 minutes prior to SDS-PAGE analysis.

4. **Sonicate the residual suspension under ice-cooling.** Take care that the suspension does not become warm or even hot which may denature proteins or activate proteases. Perform bursts with cooling intervals if possible. French pressing is possible as well.

5. **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.**

6. **Centrifuge the suspension at >15,000 x g (microfuge) for 15 minutes at 4°C.**

7. **Insoluble cell components are pelleted. If the recombinant protein forms inclusion bodies it will be present in the pellet.**

8. **Carefully transfer the cleared supernatant to a clean tube.** Store the

supernatant on ice until further usage or store at –20°C if chromatography cannot be performed on the same day. For analysis of the insoluble part of the expressed protein, dissolve the pellet with 1.25 ml 1 x SDS-PAGE sample buffer (= 250 µl 5 x SDS-PAGE sample buffer mixed with 1 ml Buffer W).

9. **Proceed to protocols for Strep-tag®II and Twin-Strep-tag® protein purification (see 4.1 to 4.3).**

3.2 Preparation of cleared lysate after periplasmic expression of Strep-tag®II and Twin-Strep-tag® fusion proteins

Periplasmic proteins are secreted into the periplasmic space located between the outer and inner membrane of *E. coli*. Proper secretion is only possible when the recombinant protein has an N-terminal signal peptide (e.g. OmpA) which is cleaved after translocation by *E. coli* leader peptidase. In order to purify proteins secreted into the periplasmic space, the Strep-tag®II or Twin-Strep-tag® can be fused either to the C- or N-terminus using e.g. pASG-IBA2, -102, -4 or -104.

Recommended Buffers/Solutions	Concentration of ingredients	Notes
Buffer P	100 mM Tris/HCl pH 8.0 500 mM sucrose 1 mM EDTA	Used for the release of the periplasmic content. It is recommended to work with 2 mg/ml polymyxin B sulfate instead of 1 mM EDTA when metalloproteins are isolated.
5x SDS-PAGE sample buffer	250 mM Tris/HCl, pH 8.0 25% glycerol 7.5% SDS 0.25 mg/ml bromophenol blue 12.5% v/v 2-mercaptoethanol	

Protocol

1. **Chill Buffer P at 4°C.**

2. **Resuspend the pellet of a 100 ml culture in 1 ml Buffer P.**

3. **Incubate 30 minutes on ice.** These conditions will usually sufficiently permeabilize the outer membrane of *E. coli* to release the soluble periplasmic components and leave the spheroplasts intact to ensure low contamination of the protein preparation with cytoplasmic proteins [10]. Harsher treatments, e.g. osmotic shock or use of lysozyme may be used if the periplasmic components are not completely released with the EDTA treatment.

4. **Collect a 10 µl sample for total analysis of the protein content via SDS-PAGE and/or Western blotting.** The 10 µl sample should be thoroughly mixed with 90 µl Buffer W and 25 µl 5 x SDS-PAGE sample buffer. Store at –20°C. The whole sample must be incubated in an ultrasonic bath for 15 minutes to shear the chromosomal DNA to small pieces and should be heated to 70°C for 10 minutes prior to SDS-PAGE analysis.

5. **Remove spheroplasts by centrifugation at 15,000 x g (microfuge) for 5 minutes at 4°C.**

6. **Carefully transfer the cleared supernatant to a clean tube.** Store the supernatant on ice until further usage or store at –20°C if chromatography cannot be performed on the same day. To check whether a part of the expressed protein remained in the cells, resuspend the sedimented spheroplasts with 1 ml Buffer P and add 250 µl 5x SDS-PAGE sample buffer and perform SDS-PAGE, optionally followed by Western blotting.

Important note

7. **Proceed to protocols for Strep-tag®II and Twin-Strep-tag® protein purification (see 4.1 to 4.3).**

4 Purification of Strep-tag® or Twin-Strep-tag® fusion proteins

To allow an efficient purification with Strep-Tactin®XT we recommend using column purification instead of batch applications. It is crucial that protein binding takes place on the column. Even a pre-incubation of resin and lysate prior to filling the resin into a column can lead to decreased protein yields. Further, prolonged batch incubations increase the risk of proteolytic degradation of the target protein including cleavage of the tag. For small volumes and high throughput applications, we recommend the use of MagStrep type 3 XT Beads (Cat.no: 2-4090-002).
For larger volumes we recommend the application of WET FRED – the applicator for Strep-Tactin® 1 ml, 5 ml and 10 ml gravity flow columns. WET FRED enables convenient application of large cell culture supernatant volumes to a gravity flow column in a simple way (Cat.no: 2-0910-001).

4.1 Purification of Strep-tag®II and Twin-Strep-tag® fusion proteins using gravity flow columns

Recommended buffers/solutions	Concentration of ingredients	Notes
Buffer W	100 mM Tris/HCl pH 8.0 150 mM NaCl 1 mM EDTA	10 x Buffer W has to be diluted before application. It is recommended to work without EDTA when metalloproteins are purified
Buffer BXT (elution buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 50 mM biotin	10 x Buffer BXT has to be diluted before application.

Important notes

- The pH should not be lower than 6.0 for Strep-Tactin®XT Superflow and 4.0 for Strep-Tactin®XT 4Flow®, respectively.
- The composition of all buffers can be modified to suit the particular application. A list with compatible reagents is available at https://www.iba-lifesciences.com.
- Strep-Tactin®XT allows purification under denaturing conditions. Therefore, up to 6 M urea can be added to all buffers. Please note, high concentrations of urea can lead to reduced yield.
- Generally, it is recommended to perform chromatography at 4°C. Dependent on the individual equipment this is not always possible and chromatography has to be performed at room temperature. If columns are stored at 4°C and transferred to room temperature air bubbles may form since cold buffers are able to take up more gas than buffers at ambient temperature. Therefore, it is recommended

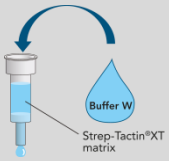
to equilibrate the columns immediately after exposure to higher temperatures with buffer that is equilibrated at such temperatures.

Recommended volumes for working with Strep-Tactin®XT columns

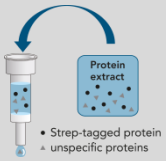
Table 1: Calculation of the required buffer volumes based on the column bed volume. The column bed volume corresponds to the amount of resin. This means a 0.2 ml gravity flow column contains a column bed volume of 0.2. Example: 6 x 1 CV buffer should be applied to a 0.2 ml column. In this case, 6 x 0.2 ml or a total of 1.2 ml buffer must be applied to the column.

n x CV	column bed volume (CV)				required buffer volume (ml)
quantity (n)	0.2	1	5	10	
0.5	0.1	0.5	2.5	5	
0.6	0.12	0.6	3	6	
0.8	0.16	0.8	4	8	
1	0.2	1	5	10	
1.6	0.32	1.6	8	16	
2	0.4	2	10	20	
5	1	5	25	50	
6	1.2	6	30	60	
8	1.6	8	40	80	
15	7.5	15	75	150	

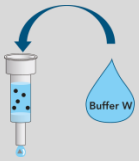
Protocol



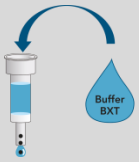
1. **Remove top cap from column first, then twist of lower cap.** If the caps are removed in reverse order, air may enter the column bed. Remove storage buffer. The column cannot run dry under gravity flow. **Equilibrate the Strep-Tactin®XT column with 2 x1 CV (column bed volume) Buffer W.**



2. **Centrifuge cleared lysate (18,000 x g, 5 minutes, 4°C).**Frozen cell extracts have to be centrifuged prior to application in order to remove any aggregates that may have formed. **Add supernatant of cleared lysate to the column.**



3. **Wash the column with 5 x 1 CV Buffer W, after the cell extract has completely entered the column.** Collect the wash fractions (1 CV each) and optionally save 2 µl of each subsequent wash fraction for application on an analytical SDS-PAGE gel.



4. **Add 6 x 0.5 CV Buffer BXT and collect the eluate in 0.5 CV fractions.** Optional: To get high protein concentrations in one fraction add 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2) and finally 0.8 CV (E3). Main protein content should be in E2. 20 µl samples of each fraction can be used for SDS-PAGE analysis.

4.2 Purification of Strep-tag®II and Twin-Strep-tag® fusion proteins on chromatography workstations using cartridges

Recommended buffers/solutions	Concentration of ingredients	Notes
Buffer W	100 mM Tris/HCl pH 8.0 150 mM NaCl 1 mM EDTA	10 x Buffer W has to be diluted before application. It is recommended to work without EDTA when metalloproteins are purified
Buffer BXT (elution buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 50 mM biotin	10 x Buffer BXT has to be diluted before application.
5x SDS-PAGE sample buffer	250 mM Tris/HCl, pH 8.0 25% glycerol 7.5% SDS 0.25 mg/ml bromophenol blue 12.5% v/v 2-mercaptoethanol	

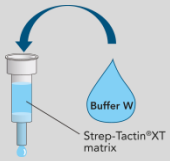
Important notes

- 1 ml and 5 ml cartridges are designed for use with chromatography workstations with 10-32 fittings (HPLC and Äkta). However, they can also be operated with other workstations, syringes or peristaltic pumps by use of appropriate adapter sets (Luer lock, cat. no. 2-1021-001; M6, cat. no. 2-1022-001; 1/4-28, cat. no. 2-1023-001; 1/16 inch, cat. no. 2-1025-001)
- Cartridges can be connected with a coupling adapter in series to enlarge capacity (Cat. no. 2-1026-001).
- Recommended flow rates: 0.5-1 ml/min for a 1 ml cartridge; 1-3 ml/min for a 5 ml cartridge.
- The pH should not be lower than 6.0 for Strep-Tactin®XT Superflow and 4.0 for Strep-Tactin®XT 4Flow®, respectively.
- The composition of all buffers can be modified to suit the particular application. A list with compatible reagents is available at https://www.iba-lifesciences.com.
- Generally, it is recommended to perform chromatography at 4°C. Dependent on the individual equipment this is not always possible and chromatography has to be performed at room temperature. If columns are stored at 4°C and transferred to room temperature air bubbles may form since cold buffers are able to take up more gas than buffers at ambient temperature. Therefore, it is recommended to equilibrate the columns immediately after exposure to higher temperatures with buffer that is equilibrated at such temperatures.
- Cartridges do not generate significant back pressure which makes the application of flow restrictors superfluous.
- Strep-Tactin®XT allows purification under denaturing conditions. Therefore, up to 6 M urea can be added to all buffers. Please note,

	high concentrations of urea can lead to reduced yield.

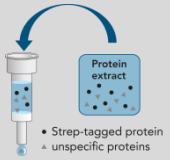
Protocol

1. **Connect adapters to the cartridge if fittings other than 10-32 are required and connect the cartridge with the chromatography workstation.**

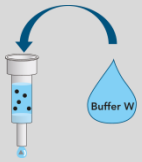


2. **Equilibrate cartridge with 5 CV (column bed volumes) of Buffer W.**
The flow rate should be in the range of 0.5–1 ml/min for 1 ml cartridges and 1–3 ml/min for 5 ml cartridges. Monitor signal at 280 nm – the baseline should be stable after washing with Buffer W.

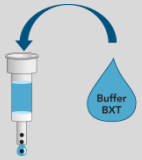
3. **Centrifuge cleared lysate (18,000 x g, 5 min, 4°C).**Frozen cell extracts have to be centrifuged prior to application in order to remove any aggregates that may have formed.



4. **Apply lysate to cartridge.**
Begin with a flow rate of 1 ml/min. Monitor pressure at this step. If the lysate is very viscous and pressure is increased significantly, reduce viscosity of the extract (please note table 1) or reduce flow rate. Collect the flow-through for SDS-PAGE analysis.



5. **Wash with Buffer W until A_{280} is stable.**
Usually 5-10 CVs are sufficient to reach the baseline. To get maximal protein yields proceed with step 5 as soon as the baseline is reached. Collect fractions for SDS-PAGE analysis.



6. **Elute protein with Buffer BXT.**
Collect fractions for SDS-PAGE analysis.

4.3 Troubleshooting – Strep-Tactin®XT purification

4.3.1 "No or weak binding to Strep-Tactin®XT resin"

pH is not correct.	• The pH should be greater than 6.0 for Strep-Tactin®XT Superflow® or pH 4.0 for Strep-Tactin®XT 4Flow®.
Twin-Strep-tag® is not present.	• Use protease deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
Twin-Strep-tag® is not accessible.	• Fuse Twin-Strep-tag® to the other protein terminus; use other linker. • Reduce washing volume to 3 CVs.
Twin-Strep-tag® has been degraded.	• Check that the Twin-Strep-tag® is not associated with a portion of the protein that is processed. Avoid purification in discontinuous batch mode. Prolonged batch incubations increase the risk of proteolytic degradation of the target protein including cleavage of the tag.
Strep-Tactin®XT column is inactive.	• Check activity with HABA.
Flow rate is too fast	• Reduced flow rates may increase yields depending on the given recombinant protein.
The column is not properly regenerated.	• Use 3 M $MgCl_2$ and increase the volume of regeneration buffer applied to the column. • Prepare fresh 10–20 mM NaOH and regenerate again. • Efficient regeneration can be visualized by addition of HABA. When HABA is added to the column it changes its color from yellow to dark orange.

4.3.2 "Contaminating proteins"

Please note: The soluble part of the *E. coli* total cell extract contains no proteins beyond the 22 kDa binding biotin carboxyl carrier protein (BCCP) which binds significantly to the Strep-Tactin®XT column. Therefore, contaminating proteins interact, specifically or non-specifically, with the recombinant protein itself and are thus co-purified.

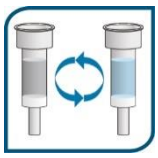
Contaminants are short forms of the tagged protein.	• Use protease deficient *E. coli* expression strains. • Add protease inhibitors after cell lysis. • Fuse the Strep-tag®II or Twin-Strep-tag® with the other protein terminus. Check for the presence of internal translation initiation starts (only in case of C-terminal Strep-tag®II or Twin-Strep-tag®) or premature termination sites (only in case of N- terminal tag). • Add 6x*Histidine*-tag to the other terminus and use both tags for purification which will lead to full length protein preparations.
Contaminants are covalently linked to the recombinant protein via disulfide bonds.	• Add reducing agents to all buffers for cell lysis and chromatography.
Contaminants are non-covalently linked to the recombinant protein.	• Increase ionic strength in all buffers for cell lysis and chromatography (up to 1 M NaCl) or add mild detergents (0.1% Triton X-100, 0.1% Tween, 0.1% CHAPS, etc.).
Contaminants are biotinylated proteins.	• Add avidin or biotin blocking buffer solution (BioLock, Cat.no: 2-0205-050) to supernatant before application onto the column.

4.3.3 Air bubbles in the column

When the column is taken from the cold storage room to the bench, the different temperatures can cause small air bubbles in the column. The reason is that the cold buffer is able to take up more gas than buffers at ambient temperature.

To prevent development of bubbles in the column bed.	Keep on working in the cold room (also recommended for proteins), use degassed buffers or wash the column immediately with buffers at ambient temperature once the column is removed from the cold.

5 Storage and regeneration of Strep-Tactin®XT resin



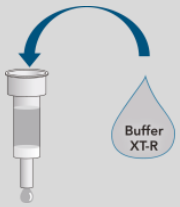
Recommended buffers/solutions	Concentration of ingredients	Notes
Buffer XT-R	3 M $MgCl_2$	For Strep-Tactin®XT, the buffer is ready to use and should not be diluted.
Buffer W	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	10 x Buffer W has to be diluted before application. It is recommended to work without EDTA when metalloproteins are purified

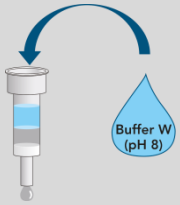
Important notes

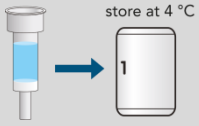
- Strep-Tactin®XT matrices should be stored in buffer W (pH 8.0) at temperatures between 4–8°C for longer terms.
- We recommend a maximum of 5 runs per column.
- Resin tolerates washing with 6 M urea. Such procedures should not last longer than 30 minutes and the resin should be equilibrated immediately with Buffer W afterwards.
- Efficient regeneration of Strep-Tactin®XT **high capacity** requires more column volumes of Buffer XT-R than Strep-Tactin®XT (see 5.3).

5.1 Regeneration of Strep-Tactin®XT gravity flow columns

Protocol







1. **Wash column with 6 CV of Buffer XT-R.** Strep-Tactin®XT resin cannot be regenerated using the hydroxy-azophenyl-benzoic acid (HABA) containing Buffer R (100 mM Tris/HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM HABA). However, after treatment with Buffer XT-R, operability can be confirmed by application of Buffer R which induces an orange-shift in case of a successful regeneration.
2. **Immediately remove the Buffer XT-R by adding 2 x 4 CV Buffer W (pH 8.0).**
3. **Store the column at 4°C overlaid with 2 ml Buffer W (pH 8.0).**

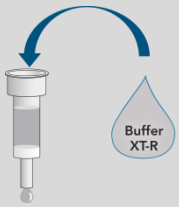
5.2 Regeneration of Strep-Tactin®XT cartridges

Protocol

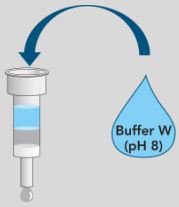
1. **Fill the cartridge inlet and the cartridge with Buffer XT-R.**
2. **Wash Strep-Tactin®XT and Strep-Tactin®XT high capacity matrices with at least 6 CVs and 15 CV Buffer XT-R, respectively. Meanwhile, the flow rate should not exceed 1 ml/min.**
   Please note: Washing with <15 CV Buffer XT-R will not completely regenerate the Strep-Tactin®XT high capacity matrices.
3. **Immediately remove the Buffer XT-R by washing with 8 CV of Buffer W (pH 8.0).**
4. **Store the cartridge at 4–8°C.**

5.3 Regeneration of Strep-Tactin®XT High Capacity gravity flow columns

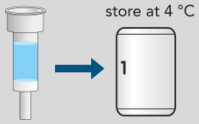
Protocol



1. **Wash the column with 15 CV of Buffer XT-R.**
   Strep-Tactin®XT high capacity resin cannot be regenerated using hydroxy-azophenyl-benzoic acid (HABA) containing Buffer R (100 mM Tris/HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM HABA). However, after treatment with Buffer XT-R, operability can be confirmed by application of Buffer R which induces an orange-shift in case of a successful regeneration.
   Please note: Washing with <15 CV Buffer XT-R will not completely regenerate the Strep-Tactin®XT high capacity matrices.



2. **Immediately remove the Buffer XT-R by adding 2 x 4 CV Buffer W (pH 8.0).**



3. **Store the column at 4°C overlaid with 2 ml Buffer W (pH 8.0).**

6 References

For up-to-date references see www.iba-lifesciences.com

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Please refer to https://www.iba-lifesciences.com for downloading this manual.

7 Notes

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