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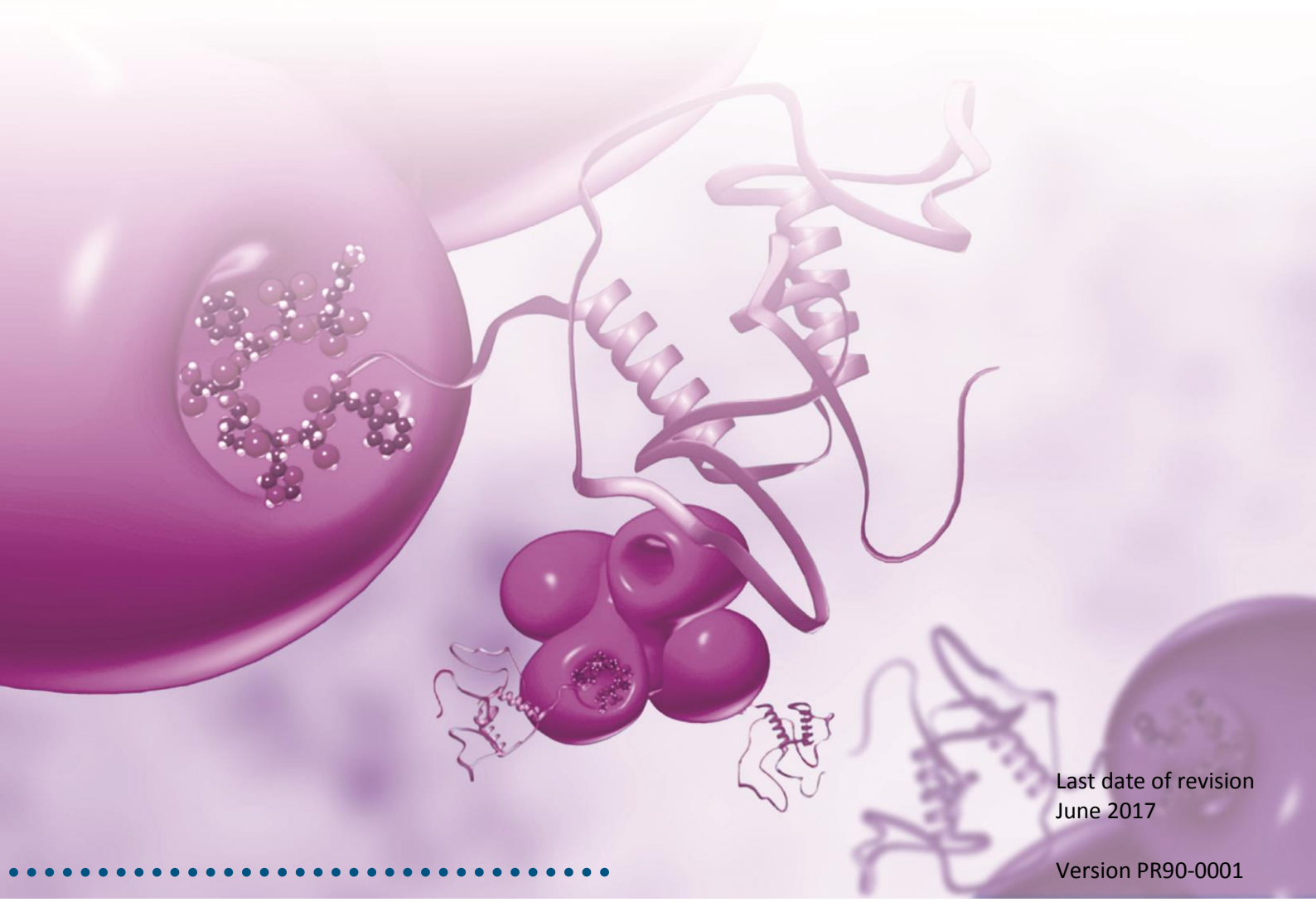
Strep-Tactin®XT Spin Column

Purification Protocol



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This manual can be downloaded under https://www.iba-lifesciences.com/download-area.html.

1 Strep-tag® system overview

The Strep-tag® II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to Strep-Tactin®, an engineered streptavidin. This technology allows one-step purification of almost any recombinant protein under physiological conditions, thus preserving its bioactivity.
The Strep-tag® system can be used to purify functional Strep-tag®II proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria.

Based on the proprietary Strep-tag® technology the **Twin-Strep-tag®** was developed which is a sequential arrangement of two Strep-tag®II sequences (28 amino acids, WSHPQFEK$(GGGS)_2$GGSAWSHPQFEK). This tag enables the same mild and rapid purification as Strep-tag®II but, due to its avidity effect, has an increased affinity for Strep-Tactin®. Like Strep-tag®II, the Twin-Strep-tag® tolerates diverse buffer conditions and additives (high salt, detergents, reducing agents, metal ions and chelating agents) making it a universal tag for varying protein properties, particularly for protein complexes in protein interaction analysis.

The latest generation of the Strep-tag® system is a further development of the commonly used Strep-Tactin®:Strep-tag®II technology. IBA's 3rd generation Strep-tag® system is based on the novel **Strep-Tactin®XT** Superflow® resin in combination with the aforementioned Twin-Strep-tag®. Strep-Tactin®XT has a **binding affinity in low pM ranges** for the Twin-Strep-tag® still maintaining the binding reversibility, the mild recovery of immobilized proteins and the high purity (> 95 %). Further, Strep-Tactin®XT now enables new applications in the field of high throughput screening, batch purification, purification using denaturing conditions and protein interaction studies making the system superior to all other available affinity tag purification systems. Strep-Tactin®XT can also be used in combination with Strep-tag®II. Those interaction partners have a binding affinity in the low nM range.
The Strep-Tactin®XT Spin Columns allow the fast and economic purification of up to 400 µg Strep-tag® protein from up to 500 µl crude extract.

A comprehensive protocol with additional information on protein expression and preparation of cleared lysates can be found in the Strep-Tactin®XT Purification Manual on our homepage https://www.iba-lifesciences.com/download-area.html .

Important notes

- The affinity matrix in the Strep-Tactin®XT Spin Columns has the same purification properties as Strep-Tactin®XT MacroPrep®.
- Since protein purification is based on the highly selective binding of Twin-Strep-tag® and Strep-tag®II to Strep-Tactin®XT, the contact time of the lysate with the resin has to be sufficient for complete complex formation. Therefore, it is important not to exceed 700 x g (approx. 2000 rpm in a microfuge) when centrifuging Strep-Tactin®XT Spin Columns for protein binding. However, wash steps should be performed at maximum speed.
- To increase protein yields the flow through can directly be re-loaded again.
- To ensure buffer flow during the centrifugation steps, the Spin Columns should be centrifuged with an open lid. For very viscous cell lysates, it may be necessary to extend the centrifugation time.
- The pH of all lysates and buffers should not be lower than 7.0.
- To prevent proteins from being degraded during cell harvest, lysis, or even purification, it is recommended to work quickly at 4°C and – if necessary – to add protease inhibitors.

2 Purification using Strep-Tactin®XT Spin Columns

Recommended Buffers/Solutions	Concentration of ingredients	Notes
Buffer W (Strep-Tactin®/ Strep-Tactin®XT Wash Buffer	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	It is recommended to work without EDTA when metalloproteins have been expressed
Buffer BXT (Strep-Tactin®XT Biotin Elution Buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 50 mM biotin	

Important notes

- The Spin Column matrix binds up to 15 nmol recombinant Strep-tag® fusion protein (corresponding to 400 µg of a 28 kDa protein (mCherry-Strep-tag®)).
- Protein yield can be increased by applying the flow through from Step 3 a second time.
- The composition of the lysis, wash and elution buffers can be modified to suit the particular application, e.g. by adding a mild detergent, a reducing reagent, protease inhibitors, glycerol or by modifying the ionic strength. The pH should not be lower than 7.0, though.
- Generally, it is recommended to perform protein purification at 4°C.
- Due to the Spin Column design it might happen that small amounts of lysate or wash buffer remain on the plastic ring fixing the column. Removal of such liquid prior to next step will achieve highest purities. Elution buffer should be applied in the center of the column.
- **Strep-Tactin®XT Spin Columns cannot be re-used.**

Protocol

1. **Centrifuge cleared lysates (13.000 rpm, 5 min, 4°C, microfuge).**
   Insoluble aggregates which may have formed after storage may clog the column and thus have to be removed.

2. **Equilibrate the Strep-Tactin®XT Spin Column with 2x 500 µl Buffer W and centrifuge for 30 seconds at 700 x g (approx. 2000 rpm) for each step and discard the flow-through.**
   This step rehydrates the dried Strep-Tactin®XT resin for the subsequent use.

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Protocol (continued)

3. **Load up to 500 µl supernatant of cleared lysate onto the pre-equilibrated Strep-Tactin®XT Spin Column. Centrifuge for 30 seconds at 700 x g (approx. 2000 rpm).**
   Collect the flow-through. Apply 2 µl to an analytical SDS-PAGE.
   Lysates with the recombinant protein at low concentration may lead to reduced yields. To increase the yield apply the flow through of step 3 and repeat this step.
   For very concentrated cell lysates, it may be necessary to extend the centrifugation time to 3-4 minutes.

4. **Wash the column 4 times with 100 µl Buffer W. Centrifuge at each step for 30 seconds at 13.000 rpm.**
   Collect the flow-through. Apply 2 µl of the first washing fraction and 20 µl of each subsequent fraction to an analytical SDS-PAGE.

5. **Place the spin column into a fresh 1.5 ml reaction tube and choose one of the following procedures for elution:**

   a. **For maximum protein yield:**
      Elute the recombinant protein by adding 3 times 150µl Buffer BXT (Strep-Tactin®XT Biotin Elution Buffer). At each step: First, centrifu ge for 30 seconds at 700 x g (approx. 2000 rpm) and finish with 15 seconds at maximum speed. Pool the eluates.

   b. **For maximum protein concentration:**
      Elute the protein with 50µl Buffer BXT (Strep-Tactin®XT Biotin Elution Buffer). First, centrifuge for 30 seconds at 700 x g (approx. 2000 rpm) and finish with 15 seconds at maximum speed. Transfer the eluate from the collection tube onto the Spin Column and repeat the centrifugation step as above to maximize the yield.

   10 µl samples of each fraction can be used for SDS-PAGE analysis.
   Biotin and EDTA can be removed, if necessary, via dialysis or gel chromatography.

3 Trouble Shooting

3.1 No or weak binding to Strep-Tactin®XT column

pH is not correct.	The pH should be > 7.0
Fusion-tag (Strep-tag®II or Twin-Strep-tag®) is not present.	Use protease deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
Fusion-tag is not accessible.	Fuse Strep-tag® with the other protein terminus; use other linker or try Twin-Strep-tag®.
Fusion-tag has been degraded.	Check that the Fusion-tag is not associated with a portion of the protein that is processed.
Fusion-tag is partially accessible.	Reduce washing volume to 2x 100 µl.
Strep-Tactin®XT column is inactivated by biotin.	Add avidin if biotin containing extracts are intended to be purified (The total biotin content of the soluble part of the total *E. coli* cell lysate is about 1 nmol per liter culture (OD_{550} = 1.0), however, it might be much higher when proteins are purified from cell culture supernatants. Add 2-3 nmol of avidin monomer per nmol of biotin.

3.2 Contaminating proteins

Note: The soluble part of the *E. coli* total cell extract contains no proteins beyond the nearly irreversibly 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, therefore, are co-purified.

Contaminants derive from remaining lysate.	Due to the Spin Column design it might happen that small amounts of lysate or washing buffer remain on the plastic ring fixing the column. Removal of such liquid prior to next step will achieve highest purities. Elution buffer should be applied in the center of the column.
Contaminants are short forms of the tagged protein.	Use protease deficient *E. coli* expression strains. Add protease inhibitors after cell lysis. Fuse Strep-tag® II with the other protein terminus. Check for the presence of internal translation initiation starts (only in case of C- terminal Strep-tag®II) or premature termination sites (only in case of N- terminal Strep-tag®II). Add 6xHis-tag to the other terminus and use both tags for purification which will lead to full length protein preparations.

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3.2 Contaminating proteins (continued)

Contaminants are covalently linked to the recombinant protein via disulfide bonds.	Add reducing reagents 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 5 M NaCl) or add mild detergents (up to 2 % Triton X-100, 2 % Tween 20, 0.1 % CHAPS, etc).

Please refer to **www.iba-lifesciences.com/technical-support.html** for downloading this manual.

Your notices

Solutions
For Life Sciences

IBA Headquarters IBA
IBA GmbH
Rudolf-Wissell-Str. 28
37079 Goettingen
Germany
Tel: +49 (0) 551-50672-0
Fax: +49 (0) 551-50672-181
E-mail: info@iba-lifesciences.com

US Stock Center
1328 Ashby Road
Olivette, MO 63132
USA
Tel. 1-877-IBA-GmbH
(1-877-422-4624)
Fax 1-888-531-6813
E-mail: info@iba-lifesciences.com