Protein Production & Assays

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Protocol

Strep-Tactin®XT

Spin Column protein purification

1. GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Components:

	Strep-Tactin®XT Spin Columns (Cat. No. 2-4150-025)	Strep-Tactin®XT Spin Column Kit (Cat. No. 2-4151-000)
Pre-packed spin columns	25 pieces	25 pieces
Receiver tubes	25 pieces	25 pieces
1x Buffer W (100 mM Tris/HCl pH 8, 150 mM NaCl, 1 mM EDTA)	Not included	120 ml
1x Buffer BXT (100 mM Tris/HCl pH 8, 150 mM NaCl, 1 mM EDTA, 50 mM biotin)	Not included	25 ml

Binding capacity: up to 400 µg of a Twin-Strep-tag® or Strep-tag®II fusion protein. The binding capacity was determined with mCherry fused to Strep-tag®II (28 kDa). Depending on the target protein properties the binding capacity can vary.

Storage: 2 - 8 °C

Stability: 6 months after shipping

Shipping: Room temperature

Warnings: Warnings are stated on the Material Safety Data Sheet.

Important information: Strep-Tactin®XT Spin Columns (Cat. No. 2-4150-025) are not provided with wash and elution buffer to allow the preparation and application of protein specific buffer compositions. Metalloproteins, for example should be purified with buffers without chelating agents, like EDTA. A list with compatible reagents for Strep-Tactin®XT protein purification can be downloaded at www.iba-lifesciences.com/download-area-protein.html. However, the Strep-Tactin®XT Spin Column Kit (Cat. No. 2-4151-000) contains wash and elution buffer for immediate protein purification. Please note, the spin columns are not reusable.

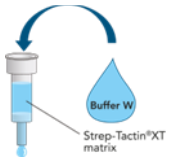
2 Description

Strep-Tactin®XT Spin Columns contain dried polymethacrylate, a synthetic material, coupled with the engineered streptavidin variant Strep-Tactin®XT and are suitable for purification of Strep-tag®II or Twin-Strep-tag® proteins from any expression system including insect cells, mammalian cells, yeast, plants and bacteria. The Strep-tag®II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to Strep-Tactin® and Strep-Tactin®XT. The Twin-Strep-tag® consists of two sequential arranged Strep-tag®II sequences (28 amino acids, WSHPQFEK-GGGS-GGGS-GGSA-WSHPQFEK) and enables the same mild purification as Strep-tag®II but, due to its avidity effect, has an increased affinity for Strep-Tactin® and Strep-Tactin®XT. Therefore, the Strep-tag® technology allows one-step purification of almost any recombinant protein under physiological conditions while preserving its bioactivity. The pre-packed spin columns are provided with receiver tubes compatible with conventional microcentrifuges allowing a rapid, parallel processing of multiple samples from small-scale expression cultures. Up to 15 nmol (400 µg of mCherry-Strep-tag®II (28 kDa)) can be purified under physiological conditions in less than 10 minutes. In contrast to agarose resins, polymethacrylate resins do not bind unspecific sample molecules, like chlorophyll, allowing mild protein purification with high purity from complex mixtures.

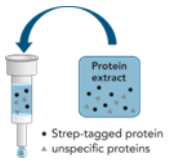
3 PROTOCOL

- The buffer composition can be modified. However, the pH should not be lower than 7.
- The contact time of the lysate with the resin has to be sufficient for complete complex formation. Therefore, the speed of the microcentrifuge should not exceed 700 x g (approx. 2000 rpm). Wash steps should be performed at maximum speed.
- The Spin Columns should be centrifuged with an open lid to ensure that the sample or buffer flow through. For very viscous cell lysates, it may be necessary to extend the centrifugation time.
- It is recommended to perform protein purification at 2 - 8 °C.
- Due to the Spin Column design it might happen that small amounts of sample or 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.
- To avoid unspecific binding or low binding efficiency, biotin or biotinylated proteins should be masked with BioLock or avidin. The protocol is provided at https://www.iba-lifesciences.com/download-area-protein.html.

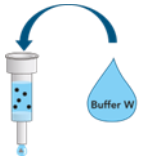
3.1 Centrifuge the cell lysate or culture supernatant (maximum speed, 5 min, 4 °C) to remove aggregates.



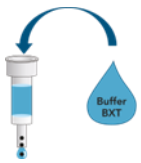
3.2 Rehydrate the Strep-Tactin®XT Spin Column twice with 500 µl 1x Buffer W and centrifuge for 30 sec at 700 x g. Discard the flow-through after each step.



3.3 Load up to 500 µl sample onto the pre-equilibrated Strep-Tactin®XT Spin Column and centrifuge for 30 sec at 700 x g. Collect the flow-through for SDS-PAGE analysis. Samples with low target protein concentrations may lead to reduced yields. Therefore, apply the flow-through and repeat this step.



3.4 Wash the column four times with 100 µl 1x Buffer W. Centrifuge for 30 sec at 15.000 x g and collect the flow-through from each step for SDS-PAGE analysis.



3.5 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 150 µl 1x Buffer BXT. Centrifuge for 30 sec at 700 x g and finish with 15 sec at maximum speed for each step. Pool the eluates.

b. For maximum protein concentration, elute the protein with 50 µl 1x Buffer BXT. Centrifuge for 30 sec at 700 x g and finish with 15 sec at maximum speed. Transfer the eluate from the collection tube onto the Spin Column and repeat the centrifugation step.

4. TROUBLESHOOTING

4.1 No or week binding to Strep-Tactin®XT column

pH is not correct.	The pH should be >7.
Strep-tag®II or Twin-Strep-tag® is not present.	Use protease deficient *E. coli* expression strains. Add protease inhibitors during cell lysis and work quickly at 2 - 8 °C
Strep-tag®II or Twin-Strep-tag® is not accessible.	Fuse the tag with the other protein terminus, use a different linker, or exchange the Strep-tag®II by Twin-Strep-tag®.
Strep-tag®II or Twin-Strep-tag® has been degraded.	Check if the tag is associated with a portion of the protein that is processed. If it is the case, change the position of the tag.
Strep-tag®II or Twin-Strep-tag® is partially accessible.	Reduce washing volume to 2 x 100 µl.
Binding capacity is reduced by biotin or biotinylated proteins.	Add our biotin blocking solution, BioLock, or avidin. The protocol for biotin blocking can be downloaded at https://www.iba-lifesciences.com/download-area-protein.html.
Increase the protein yield	Re-load the flow-through again prior to the wash step.

4.2 Contaminating proteins

	The soluble part of the E. coli total cell extract does not contain proteins beyond the 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.	Small amounts of the sample can remain on the plastic ring fixing the column. Remove the remaining sample from the ring prior to the next step. 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 if internal translation initiation starts (only in case of C- terminal tag) or premature termination sites (only in case of N- terminal tag) are present. Add another 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 reagents to all buffers necessary for cell lysis and chromatography.
Contaminants are non-covalently linked to the recombinant protein.	Increase the ionic strength of all buffers (up to 5 M NaCl) or add mild detergents (up to 2% Triton X-100, 2% Tween 20, 0.1% CHAPS, etc). A list with compatible reagents can be downloaded at https://www.iba-lifesciences.com/download-area-protein.html.
Contaminants are biotinylated proteins.	Add biotin blocking solution, BioLock, or avidin. A detailed protocol for biotin blocking can be downloaded at https://www.iba-lifesciences.com/download-area-protein.html.

Check our Downloads page

www.iba-lifesciences.com/download-area.html

for the latest version of this manual.

Info on warranty / licensing and trademarks available at:

www.iba-lifesciences.com/patents-licenses-trademarks.html

If you have any questions, please contact

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We are here to help!

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