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Protocol

Strep-Tactin®XT 4Flow® high capacity Spin Column Kit

For fast and simply protein purification of multiple small samples

1 GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Cat. No.: 2-5151-000

Kit Components:

Strep-Tactin®XT 4Flow® high capacity resin	4 ml 50% suspension
Empty spin columns	40 pieces
1x Buffer W	120 ml (100 mM Tris/HCl pH 8, 150 mM NaCl, 1 mM EDTA)
1x Buffer BXT	25 ml (100 mM Tris/HCl pH 8, 150 mM NaCl, 1 mM EDTA, 50 mM biotin)

Binding capacity: Based on a column bed volume of 50 µl up to 1550 µg of a Twin-Strep-tag® fusion protein can be purified. The binding capacity was determined with a 50 kDa protein fused to Twin-Strep-tag®. Depending on the target protein properties the binding capacity can vary.

Storage: 2-8 °C

Stability: 6 months after shipping

Shipping: Room temperature

Important information: The Strep-Tactin®XT 4Flow® high capacity Spin Column Kit (Cat. No. 2-5151-000) contains Strep-Tactin®XT 4Flow® high capacity resin, empty spin columns, wash and elution buffer for immediate protein purification. Please note that the regeneration of the resin after the spin column procedure is incomplete and, therefore, the resin should not be reused. In addition, empty spin columns (Cat. No. 2-5150-050) without buffer and resin are provided to facilitate the free design of experiments. 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

Further material Please note that receiver tubes are not included. As receiver tubes, conventional 1.5-2 ml reaction tubes are compatible.

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

2 DESCRIPTION

Strep-Tactin®XT 4Flow® high capacity is a 4% agarose coupled with the engineered streptavidin variant Strep-Tactin®XT. In combination with spin columns, it is suitable for fast and easy purification of Strep-tag®II or Twin-Strep-tag® proteins from small sample volumes and any expression system including insect cells, mammalian cells, yeasts, 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. Strep-Tactin®XT 4Flow® high capacity Spin Columns are not pre-packed, allowing an adjustment of the resin bed volume depending on the amount of protein to be purified. Up to 20 nmol protein (600 µg of mCherry-Twin-Strep-tag (30 kDa)) can be purified under physiological conditions in less than 15 minutes.

3 PROTOCOL

- 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 or dilute the sample in 1x Buffer W.
- 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 at the border of the column. Removal of such liquid prior to the next step will achieve highest purities. Elution buffer should be applied in the center of the column.
- The maximum filling volume of the spin columns is 700 µl.
- Unspecific binding of biotinylated proteins can be avoided by application of BioLock or avidin. The protocol is provided at https://www.iba-lifesciences.com/download-area-protein.html.

3.1 Resuspend Strep-Tactin®XT 4Flow® high capacity resin and pipet 100 µl of the 50% suspension into a spin column leading to a column bed volume of 50 µl.

3.2 Centrifuge the sample (maximum speed, 5 min, 4 °C) to remove aggregates.

3.3 Apply up to 500 µl sample to the spin column, close the column lid and incubate at room temperature with constant movement (rolling or shaking) for 5-30 min. For most purposes, 5 min are sufficient, but a longer incubation can increase the amount of captured protein, especially for large proteins (> 90 kDa).

3.4 After incubation, open the lid and break of the lower column seal. Place the spin column into a reaction tube and centrifuge for 30 sec at 500 x g.

3.5 Collect the flow-through for SDS-PAGE analysis and place the spin column into a new reaction tube. Apply 500 µl 1x Buffer W and centrifuge for 30 seconds at 700 x g. (Optional: Usually, one washing step is sufficient to obtain a highly pure protein, but this step can be repeated if higher purity is required.)

3.6 Collect the washing fraction for SDS-PAGE analysis and place the spin column into a new reaction tube. Proceed with elution step a or b:

a. For fast processing, apply 150-200 µl 1x Buffer BXT, close the spin column lid and vortex briefly. After 5 minutes of incubation without movement, vortex again briefly, open the spin column lid and centrifuge for 30 seconds at 700 x g.

b. For maximum target protein concentration, apply 50-100 µl 1x Buffer BXT, close the spin column lid and vortex briefly. After 5 minutes of incubation without movement, vortex again briefly, open the spin column lid and centrifuge for 30 seconds at 700 x g. Repeat this step one to two times. At least 80% of the target protein will be in the first elution fraction.

4 TROUBLESHOOTING

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

Strep-tag®II or Twin-Strep-tag® is not present.	Add protease inhibitors during cell lysis and work quickly at 2-8 °C. If *E. coli* is used as expression host, use a protease deficient expression strain.
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.

4.2 Contaminating proteins

Contaminants derive from remaining lysate.	Check the column side and remove any remaining sample before proceeding to the next step.
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.
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

https://www.iba-lifesciences.com/downloadcenter/

for the latest version of this manual.

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If you have any questions, please contact

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We are here to help!

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