Protein purification with Strep-Tactin®XT resins

Efficient protein purification with gravity flow columns

1. Description

Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity consist of a 4% agarose coupled with the streptavidin variant Strep-Tactin®XT. Strep-Tactin®XT specifically interacts via the engineered biotin binding pocket with the Strep-tag®II as well as the Twin-Strep-tag® and has the highest affinity for both tags (nM range for Strep-tag®II and pM-range Twin-Strep-tag®). Due to the specific and tight binding, target proteins can be purified with an incomparable high purity even from samples with a low target protein concentration and independent from the protein class. In comparison to Strep-Tactin®XT 4Flow®, Strep-Tactin®XT 4Flow® high capacity is coated with a Strep-Tactin®XT at a higher density leading to a higher protein binding capacity and avidity.

The elution of the target proteins occurs under mild conditions with a specific competitor, biotin, which releases the tagged target protein from the engineered biotin binding pocket without influencing the target protein's properties. If necessary, biotin can be easily removed via dialysis, size exclusion chromatography or cross flow ultrafiltration after purification.

2. General information & required material

The protocol is intended for gravity flow column-based protein purification with Strep-Tactin®XT 4Flow® or Strep-Tactin®XT 4Flow® high capacity. Prepacked gravity flow columns are offered by Cube Biotech. For customer specific gravity flow columns, Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity are also available as 50% suspension. 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 batch purifications, we recommend the application of MagStrep® Strep-Tactin®XT beads.

All necessary buffers for protein purification and subsequent regeneration of the resin are listed in the following table. Cube Biotech provides them as tenfold concentrated stock solutions, except Buffer XT-R. Due to the high concentration of $MgCl_2$, Buffer XT-R is only available as ready-to-use solution. Instead of Buffer XT-R, freshly prepared 100 mM NaOH can be used as an alternative regeneration solution but is not offered as a product by Cube Biotech.

Buffers/solutions		Cat. No.	Quantity
Wash buffer	Buffer W (10 x) (1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, pH 8)	2-1003-100	100 ml
Elution buffer	Buffer BXT (10 x) (1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, 500 mM biotin, pH 8)	2-1042-025	25 ml
Regeneration buffer	Buffer XT-R (3 M $MgCl_2$)	2-1045-250	250 ml

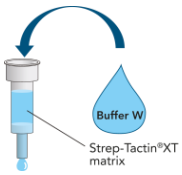
The composition of all purification buffers can be modified to suit the properties of the target protein. A list with compatible reagents is available at https://www.iba-lifesciences.com/download-area-protein.html. Please note that the resin is stable at pH 4-10.

If the target protein should be purified from larger sample volumes, we recommend the application of the WET FRED – an application aid for Strep-Tactin® and Strep-Tactin®XT gravity flow columns. The WET FRED enables convenient application of large sample volumes, e.g., cell culture supernatant, to a gravity flow column in a simple way (for 1 ml gravity flow columns Cat. No. 2-0911-001; for 5-10 ml gravity flow columns Cat. No. 2-9010-001) and is re-usable.
Usually, protein purification and binding capacity of Strep-Tactin®XT resins are not influenced by free biotin for example in cell culture supernatants, but the co-purification of biotinylated proteins is possible. Biotinylated proteins are only present in the cell in very small amounts, but if a highly pure target protein for analytic applications like mass spectrometry is required, co-purification of biotinylated proteins can be avoided by application of BioLock containing avidin. Avidin specifically masks biotinylated proteins without influencing the binding properties of the Twin-Strep-tag® or Strep-tag®II. The protocol for masking biotinylated proteins is provided at https://www.iba-lifesciences.com/download-area-protein.html.

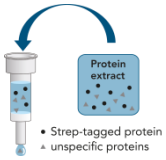
3. Protocol

3.1. Gravity flow column-based protein purification

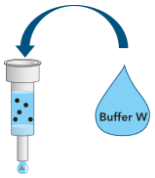
A video of the protocol using a Strep-Tactin®XT 4Flow® gravity flow column as an example is available at https://www.youtube.com/watch?v=GMGPtco43dk.



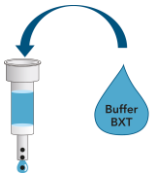
3.1.1. It is recommended to perform protein purification at **2-8 °C**. Remove the top cap from the column first, then twist off the lower cap. If the caps are removed in reverse order, air may enter the column bed. Let the storage buffer flow through. The column cannot run dry under gravity flow. Equilibrate the Strep-Tactin®XT 4Flow® column with **2x 1 CV** (column bed volume) **1x Buffer W**.
The column bed volume corresponds to the amount of resin in the gravity flow column. This means that a 0.2 ml gravity flow column contains a column bed volume of 0.2 and, therefore, 0.4 ml 1x Buffer W has to be applied.



3.1.2. Centrifuge the sample **(40,000 x g, 15 min, 4 °C**) to remove any aggregates that may have formed. Add the sample to the column and let it completely flow through by gravity flow.
Optional: Collect the flow through for subsequent SDS-PAGE analysis or for a second purification round to improve protein yield.



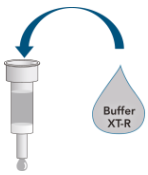
3.1.3. Wash the column with **5x 1 CV 1x Buffer W**.
Optional: Collect the five washing fractions in separate tubes for subsequent SDS-PAGE analysis



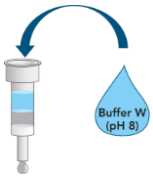
3.1.4. Add **6x 0.5 CV 1x Buffer BXT** and collect the eluate in 0.5 CV fractions. Optional: To obtain most of the eluted protein in one fraction with a high concentration add 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2), and finally 0.8 CV (E3). The main protein content should be in E2. Analyze protein purification results by SDS-PAGE.

3.2. Regeneration and storage of the gravity flow column

- Efficient regeneration of Strep-Tactin®XT 4Flow® high capacity requires more column volumes of Buffer XT-R than Strep-Tactin®XT 4Flow®. Washing of Strep-Tactin®XT 4Flow® high capacity with < 15 CV Buffer XT-R will not completely regenerate the resin.
- Freshly prepared **100 mM NaOH** can be used as an alternative regeneration solution, following the same steps as with Buffer XT-R.



3.2.1. In case of **Strep-Tactin®XT 4Flow®**: Wash the column with **6 CV** of **Buffer XT-R**.
In case of **Strep-Tactin®XT 4Flow® high capacity**: Wash the column with **15 CV** of **Buffer XT-R**.
Strep-Tactin®XT resins cannot be regenerated using 1x Buffer R containing HABA (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 1x Buffer R which induces an orange-shift in case of a successful regeneration.



3.2.2. Immediately wash the column by adding **8 CV 1x Buffer W.**

3.2.3. Overlay the column with **2 ml 1x Buffer W**. Close the column with the top cap and then with the lower cap. Store the column at **2-8 °C**.
Optional: Storage in 20% Ethanol is possible for 12 months without loss of performance.

4. Troubleshooting

No or weak binding to Strep-Tactin®XT column	
pH is not correct	The resin is stable at pH 4-10.
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. 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-tag®II or Twin-Strep-tag® is partially accessible.	Reduce the washing volume
Strep-Tactin®XT column is inactive.	Check the column activity apply HABA (1x Buffer R). Color change from yellow to red displays the regeneration process. The intensity of the red color is an indicator of the column activity status. The red color on the bottom of the column should have the same intensity as on top of the column. Remove HABA afterwards with 100 mM NaOH according to the regeneration protocol (section 3.2). Immediately afterwards, wash with 1x Buffer W as long-term exposure to 100 mM NaOH may be detrimental to the resin.
The column is not properly regenerated.	Increase the volume of Buffer XT-R applied to the column or prepare fresh 100 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 orange
Contaminating proteins	
Contaminants derive from remaining lysate.	Check the column side and remove any remaining sample before proceeding with the next step.
Contaminants are short forms of the tagged protein	When working with *E. coli*, use protease deficient expression strains. Add protease inhibitors before/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 protein purification.

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.).
Contaminants are biotinylated proteins.	Add BioLock, a biotin blocking solution containing avidin.

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 can take up more gas than buffers at ambient temperature. Generally, it is recommended performing chromatography at 2-8 °C. Dependent on the individual equipment this is not always possible, and protein purification has to be performed at room temperature. If the protein purification occurs at room temperature, use degassed buffers, and wash the column immediately with buffers at ambient temperature once the column is removed from the cold.