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Protocol

Protein purification with Strep-Tactin®XT FPLC columns

Automated purification of Strep-tag®II and Twin-Strep-tag® fusion proteins using Strep-Tactin®XT FPLC columns

1 DESCRIPTION

Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity FPLC columns are available with a 1 and 5 ml column bed volume and are intended for the purification of Strep-tag®II and Twin-Strep®-tag fusion proteins with HPLC/FPLC devices, such as Äkta® systems (Cytiva). Strep-Tactin®XT specifically interacts with Strep-tag®II as well as Twin-Strep-tag® via the engineered biotin binding pocket and has the highest affinity for both tags (nm range for Strep-tag®II and pM-range for 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.

Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity have another advantage compared to other protein purification resins. The low concentrated 4Flow® agarose facilitates the penetration and thus the capture of large proteins, which leads to high yields even for those proteins that higher concentrated agaroses cannot efficiently bind.

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.

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2 GENERAL INFORMATION AND REQUIRED MATERIAL

Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity FPLC columns contain a 4% agarose coupled with the streptavidin variant Strep-Tactin®XT. The FPLC columns are applicable for purification of Strep-tag®II or Twin-Strep-tag® fusion proteins via all common liquid chromatography instruments (including Äkta™ FPLC's), peristaltic pumps and syringes.

2.1 Recommended Buffers

All necessary buffers for protein purification and subsequent regeneration of the resin are listed in the following table. IBA Lifesciences 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 IBA Lifesciences.

Buffer/Solution	Concentration	Storage and notes
1x Buffer W	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	Wash buffer for Strep-Tactin®XT and Strep-Tactin® resins. Store at 2-8 °C.
1x Buffer BXT	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 50 mM biotin	Elution buffer for Strep-Tactin®XT resins. Store at 2-8 °C.
Buffer XT-R	3 M $MgCl_2$	Regeneration buffer for Strep-Tactin®XT resins. Store at 2-8 °C.

However, 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 pH value of the buffer should always be between 4-10.

2.2 Biotin Blocking

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.

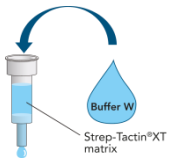
2.3 Air bubbles in the column

It is recommended to perform protein purification at 2-8 °C. Depending on the individual equipment this is not always possible, and chromatography has to be performed at room temperature. If FPLC columns are stored at 2-8 °C and are transferred to room temperature air bubbles may form due to restricted solubility of air at elevated temperatures. Therefore, it is recommended to equilibrate the FPLC columns immediately after exposure to higher temperatures with buffer that is equilibrated at the working temperature. Since FPLC columns do not generate significant back pressure, IBA recommends not using flow restrictors to avoid inhomogeneities resulting from buffer changes during chromatography.

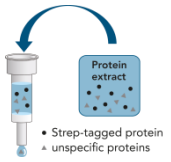
3 PROTOCOL

3.1 Purification of Strep-tag®II and Twin-Strep-tag® fusion proteins on chromatography workstations using Strep-Tactin®XT FPLC columns

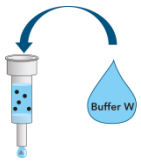
Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity FPLC columns are compatible with all common liquid chromatography instruments, such as Äkta® systems, and can be directly connected to the chromatography workstation. If fittings other than 10-32 are required, connect adapters to the FPLC column beforehand.



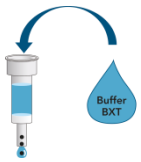
3.1.1 Equilibrate FPLC column with 5 CVs (column bed volumes) of 1x Buffer W. The flow rate should be in the range of 0.5-1 ml/min for 1 ml FPLC columns and 1-3 ml/min for 5 ml FPLC columns. Monitor elution at 280 nm; the baseline should be stable after washing with 5 CVs.



3.1.2 Apply sample to FPLC column. Begin with a flow rate of 1 ml/min. Monitor pressure at this step. If the sample is very viscous and pressure is increased significantly, reduce viscosity of the sample by dilution with 1x Buffer W or reduce flow rate. Collect the flow-through for SDS-PAGE analysis.



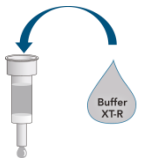
3.1.3 Wash with 1x Buffer W until A_{280} is stable. Usually, 5-10 CVs are sufficient to reach the baseline. Collect fractions for SDS-PAGE analysis.



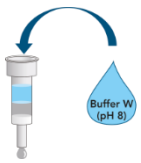
3.1.4 Elute the protein with 1x Buffer BXT until baseline at A_{280} is reached. Collect fractions for SDS-PAGE analysis.

3.2 Regeneration and storage of the FPLC column

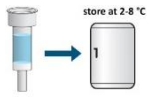
- Efficient regeneration of Strep-Tactin®XT 4Flow® high capacity requires more column volumes of Buffer XT-R than Strep-Tactin®XT 4Flow®. Washing of Strep-Tactin®XT 4Flow® high capacity with <15 CV Buffer XT-R will not completely regenerate the resin.
- Strep-Tactin®XT resins cannot be regenerated using 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.
- 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 Fill the inlet and the FPLC column with Buffer XT-R.
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. The flow rate should not exceed 1 ml/min.



3.2.2 Immediately remove the Buffer XT-R by washing with 8 CV of 1x Buffer W.



3.2.3 Store the FPLC column in 1x Buffer W at 2–8 °C.
Optional: Storage in 20 % Ethanol for 6 months is possible without loss in performance

4 TROUBLESHOOTING

4.1 No or weak binding to Strep-Tactin®XT FPLC column

pH is not correct	The pH of the sample and the buffers should be between 4-10 for Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity.
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 washing volume to 3 CVs.
Strep-Tactin®XT FPLC 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.
Flow rate is too fast.	Reduced flow rates may increase yields depending on the given recombinant protein.

4.2 Contaminating proteins

Contaminants derive from remaining lysate.	Check the FPLC column and remove any remaining sample before proceeding with 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 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 biotin blocking solution, BioLock, or avidin.

Check our Downloads page

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

for the latest version of this manual.

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https://www.iba-lifesciences.com/patents-licenses-trademarks/

If you have any questions, please contact

strep-tag@iba-lifesciences.com

We are here to help!