Protein Production & Assays

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Protocol

Protein purification with Strep-Tactin® 4Flow® high capacity FPLC columns

Automated purification of Strep-tag®II and Twin-Strep-tag® fusion proteins using Strep-Tactin® 4Flow® high capacity FPLC columns

1 DESCRIPTION

Strep-Tactin® 4Flow® high capacity FPLC columns are available with 1 or 5 ml column bed volume and intended for purification of Strep-tag®II and Twin-Strep-tag® fusion proteins with HPLC/FPLC devices, such as Äkta™ systems (Cytiva). Strep-Tactin® 4Flow® high capacity consists of a 4% agarose coated with Strep-Tactin®. The low concentrated agarose 4Flow® achieves higher yields for large proteins compared to a 6% agarose and is characterized by high pressure stability. Strep-Tactin® specifically interacts with the Strep-tag®II as well as the Twin-Strep-tag® via the engineered biotin binding pocket. It has an affinity in the µM range for Strep-tag®II and nM range for Twin-Strep-tag®. Tagged target proteins can be purified from any expression system including insect cells, mammalian cells, yeast, and bacteria, while retaining their biological activity. Due to the highly specific interaction of Strep-tag®II and Twin-Strep-tag® with Strep-Tactin®, target proteins are eluted with high purity.

The elution of the target proteins is performed by the addition of desthiobiotin in low concentrations. Desthiobiotin is a specific competitor which releases the tagged target protein from the engineered biotin binding pocket without influencing the target protein's properties. If necessary, desthiobiotin can be removed via dialysis or gel chromatography. After elution with desthiobiotin, Strep-Tactin® 4Flow® high capacity FPLC columns can be regenerated with 100 mM NaOH.

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2 GENERAL INFORMATION AND REQUIRED MATERIAL

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

2.1 Recommended buffers

All necessary buffers for protein purification are listed in the following table. IBA Lifesciences provides them as tenfold concentrated stock solutions except the regeneration solution (100 mM NaOH).

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 E	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 2.5 mM desthiobiotin	Elution buffer for Strep-Tactin® resins. Store at 2-8 °C.
Regeneration solution	100 mM NaOH	Regeneration solution for Strep-Tactin® 4Flow® high capacity resin. Always use freshly prepared NaOH solution.

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 be between 7-8 during protein purification.

2.2 Biotin blocking

Eukaryotic cultivation media (for mammalian, insect cell or yeast expression) may contain significant amounts of biotin. Biotin binds with high affinity to Strep-Tactin®, thereby efficiently competing binding of Strep-tag®II and Twin-Strep-tag®. This bond is nearly irreversible, prevents binding of the Strep-tag® fusion protein and does not allow regeneration of the Strep-Tactin® 4Flow® high capacity FPLC column (in contrast to bound desthiobiotin). Therefore, it has to be removed or masked prior to affinity chromatography. The best and simplest precaution is to add stochiometric amounts of BioLock containing avidin for irreversible masking prior to protein purification. Other solutions are removal via dialysis, ammonium sulfate precipitation or cross-flow filtration/concentration. The protocol for masking biotin or biotinylated proteins is provided at https://www.iba-lifesciences.com/download-area-protein.html.

2.3 Recommended sample volumes

If the target protein should be purified from larger sample volumes, we recommend the use of Strep-Tactin®XT 4Flow® and Strep-Tactin®XT 4Flow® high capacity, which offer a superior immobilization of target proteins. Due to their higher affinity to Strep-tag®II and Twin-Strep-tag®, target proteins do not rinse out even if large sample volumes are applied. However, Strep-Tactin® resins can still be used if the recommended volumes in the following table are considered.

Column bed volume (CV)	Sample volume*		Wash buffer volume
	Strep-tag®II	Twin-Strep-tag®	
1 ml	0.5-10 ml	0.5-100 ml	5-10 CV
5 ml	2.5-50 ml	2.5-500 ml	5-10 CV

*Adjust sample volume according to binding capacity of the column and apply it as concentrated as possible in the recommended volume range. Note that these volumes are average values which can be different for certain proteins.

2.4 Air bubbles in the column

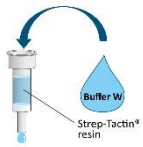
It is recommended to perform protein purification at 2-8 °C. Depending on the individual equipment this is not always possible, and chromatography has to be performed at room temperature. If FPLC columns are stored at 2-8 °C and are transferred to room temperature, air bubbles may form due to restricted 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 inhomogeneity's resulting from buffer changes during chromatography.

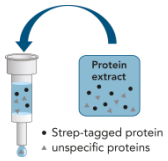
3 PROTOCOL

3.1 Protein purification using Strep-Tactin® 4Flow® high capacity FPLC columns with chromatography systems

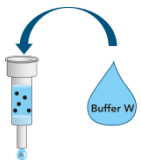
Strep-Tactin® 4Flow® high capacity FPLC columns are compatible with all common liquid chromatography instruments (like Äkta® systems) and can be directly connected to the chromatography workstation. If fittings other than 10-32 are required, connect adapters to the FPLC column beforehand.



3.1.1 Equilibrate FPLC column with at least 5 CVs (column bed volumes) 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 the flow through at 280 nm. The baseline should be stable after washing with 1x Buffer W.



3.1.3 Centrifuge the sample (18,000 x g, 5 min, 4 °C) to remove any aggregates that may have formed. Apply sample to FPLC column. Begin with a flow rate of 1 ml/min. Monitor pressure at this step. If the lysate is very viscous and pressure is increased significantly, reduce viscosity of the extract by dilution with 1x Buffer W (please note the recommended volumes for working with Strep-Tactin® 4Flow® high capacity FPLC columns in chapter 2) or reduce flow rate. Collect the flow-through for SDS-PAGE analysis.



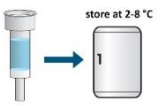
3.1.4 Wash with 1x Buffer W until A_{280} is stable. Usually, 5-10 CVs are sufficient to reach the baseline. To get maximal protein yields proceed with the next step as soon as the baseline is reached. Collect fractions for SDS-PAGE analysis.

3.1.5 Elute the protein with 5-10 CVs 1x Buffer E. Collect fractions for SDS-PAGE analysis.

3.2 Regeneration and storage of the FPLC column

3.2.1 Fill the FPLC column inlet and the FPLC column with freshly prepared 100 mM NaOH. Wash with 15 CVs 100 mM NaOH at a flow rate of 1 ml/min.

3.2.2 Immediately afterwards, exchange the FPLC column buffer to 1x Buffer W as long-term exposure to 100 mM NaOH may be detrimental to the resin. Wash with 15 CVs 1x Buffer W.



3.2.3 Store the FPLC column at 2-8 °C.

4 TROUBLESHOOTING

4.1 No or weak binding to Strep-Tactin® 4Flow® high capacity FPLC column

pH is not correct	The pH should be between pH 7-8.
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 or use a Strep-Tactin®XT resin.
Strep-Tactin® 4Flow® high capacity FPLC column is inactive.	To check the column activity, apply HABA (1x Buffer R). The 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. To avoid inactivation of the FPLC column due to biotin/biotinylated proteins, add avidin or BioLock to the cell lysate, if biotin containing extracts are intended to be purified.
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 side 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% Tergitol, 2% Tween 20, etc.).
Contaminants are biotinylated proteins.	Add avidin or BioLock, a biotin blocking solution containing avidin.

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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