Protein Production & Assays

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Protocol

Protein purification with Strep-Tactin® FPLC columns

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

1 DESCRIPTION

Strep-Tactin® Superflow® 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). The FPLC columns have a dynamic binding capacity of 7 mg protein per ml resin. 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® FPLC columns can be regenerated with HABA. It displaces desthiobiotin from the binding pocket and changes to a red color once it binds to the binding site, indicating the activity status of the resin.

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2 GENERAL INFORMATION AND REQUIRED MATERIAL

Strep-Tactin® Superflow® high capacity FPLC columns contain a 6% agarose coupled with a higher density of the streptavidin variant Strep-Tactin® compared to Strep-Tactin® Superflow®. 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.

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.
1x Buffer R	100 mM Tris/HCl pH 8.0 150 mM NaCl, 1 mM EDTA 1 mM HABA (hydroxy-azophenyl-benzoic acid)	Regeneration buffer for Strep-Tactin® 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 be between 7-8.

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® 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 leach 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	Elution buffer volume
	Strep-tag®II	Twin-Strep-tag®		
1 ml	0.5-10 ml	0.5-100 ml	5 x 1 ml	6 x 0.5 ml
5 ml	2.5-50 ml	2.5-500 ml	5 x 5 ml	6 x 2.5 ml

*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.

Binding capacity is protein dependent and normally lies between 25 and 230 nmol recombinant protein per ml bed volume (e.g., 100 nmol of mCherry-Twin-Strep-tag (~30 kDa) correspond to 3 mg protein). FPLC columns may be connected in series to increase the capacity.

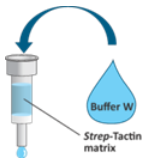
2.4 Air bubbles in the column

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.

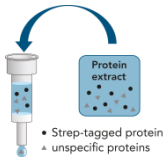
3 PROTOCOL

3.1 Protein purification using Strep-Tactin® Superflow® high capacity FPLC columns with chromatography workstations

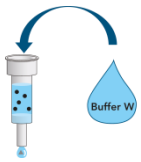
Strep-Tactin® Superflow® high capacity FPLC columns are compatible with all common liquid chromatography instruments (like Äkta® systems) and can be directly connected to the chromatography workstation. If fittings other than 10-32 are required, connect adapters to the FPLC column beforehand.



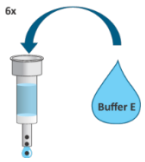
3.1.1 Equilibrate FPLC column with 5 CVs (column bed volumes) of 1x Buffer W. The flow rate should be in the range of 0.5-1 ml/min for 1 ml FPLC columns and 1-3 ml/min for 5 ml FPLC columns. Monitor the flow through at 280 nm. The baseline should be stable after washing with 5 CVs.



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® FPLC columns in chapter 2) or reduce flow rate. Collect the flow-through for SDS-PAGE analysis.

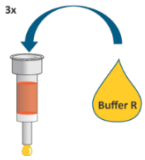


3.1.4 Wash with 1x Buffer W until A_{280} is stable. Usually, 5-10 CVs are sufficient to reach the baseline. To get maximal protein yields proceed with the next step as soon as the baseline is reached. Collect fractions for SDS-PAGE analysis.

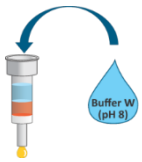


3.1.5 Elute the protein with 1x Buffer E. Collect fractions for SDS-PAGE analysis.

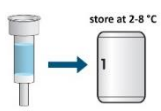
3.2 Regeneration and storage of the FPLC column



3.2.1 Fill the FPLC column inlet and the FPLC column with 1x Buffer R.



3.2.2 Wash with 15 CVs 1x Buffer R at a flow rate of 1 ml/min. Regeneration is complete when the red color on the bottom of the FPLC column has the same intensity as on top of the FPLC column. If this is not the case, use more 1x Buffer R.



3.2.3 Use 4 CV 1x Buffer W at pH 10.5 for HABA removal. Immediately afterwards, exchange the FPLC column buffer to 1x Buffer W pH 8.0 as long-term exposure to pH 10.5 may be detrimental to the resin. Store the FPLC column at 2-8 °C.

4 TROUBLESHOOTING

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

pH is not correct	The pH should 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 resin with Strep-Tactin®XT.
Strep-Tactin®XT FPLC column is inactive.	Check activity with HABA. 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.
Batch purification is carried out	To allow an efficient Strep-tag®II/Strep-Tactin® binding, it is crucial that protein binding takes place on the FPLC column. Even a pre-incubation of resin and lysate prior to filling the resin into a FPLC column will lead to decreased protein yields. If batch purification with the Strep-tag® system is intended, the use of MagStrep “type3” XT beads in combination with the Twin-Strep-tag® is recommended.
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% Triton X-100, 2% Tween 20, 0.1% CHAPS, etc.).
Contaminants are biotinylated proteins.	Add 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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