Protein purification with Strep-Tactin® FPLC columns

Automated purification of Strep-tag®II and Twin-Strep-tag® fusion proteins

1. Description

PureCube HiCap Strep-Tactin® 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). PureCube HiCap Strep-Tactin® consists of a 4% agarose coated with Strep-Tactin®. The low concentrated agarose 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, PureCube HiCap Strep-Tactin® FPLC columns can be regenerated with 100 mM NaOH.

2. General information & required material

PureCube HiCap Strep-Tactin® 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

Buffers/solutions		Cat. No.	Quantity
Wash buffer	Buffer W (100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8)	2-1003-100 (available as 10x concentrated stock solution)	100 ml
Elution buffer	Buffer E (100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8)	2-1000-025 (available at IBA Lifesciences as 10x concentrated stock solution)	25 ml
Regeneration solution	100 mM NaOH	Not provided. Prepare freshly.	

However, the composition of all purification buffers can be modified to suit the properties of the target protein. A list of 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 PureCube HiCap Strep-Tactin® FPLC columns (in contrast to bound desthiobiotin). Therefore, it must 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	20.5-100 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.

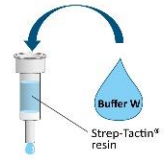
2.4. Air bubbles in the column

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, Cube Biotech recommends not using flow restrictors to avoid inhomogeneity's resulting from buffer changes during chromatography.

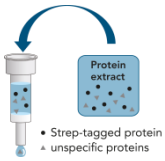
3. Protocol

3.1. FPLC column-based protein purification

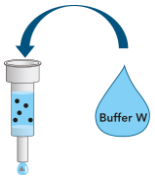
PureCube HiCap Strep-Tactin® FPLC columns are compatible with all common liquid chromatography instruments, such as Äkta® systems, and can be directly connected to the chromatography workstation. If fittings other than 10 32 are required, connect adapters to the FPLC column beforehand.



3.1.1. Equilibrate FPLC column with at least **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 1x Buffer W.



3.1.2. 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 sample is very viscous and pressure is increased significantly, reduce viscosity of the sample by dilution with 1x Buffer W (please note the recommended volumes for working with PureCube HiCap Strep-Tactin® FPLC columns in chapter 2) or reduce flow rate. Collect the flow-through for SDS-PAGE analysis.

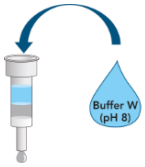


3.1.3. Wash with **1x Buffer W** until A_{280} is stable. Usually, 5-10 CVs are sufficient to reach the baseline. To get maximal protein yields proceed with the next step as soon as the baseline is reached. Collect fractions for SDS-PAGE analysis.

3.1.4. **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 in 1 CV 1x Buffer W at 2–8 °C.
Optional: Storage in 20 % Ethanol is possible without loss in performance.

4. Troubleshooting

No or weak binding to Strep-Tactin® 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 the washing volume or use a Strep-Tactin®XT resin.
Strep-Tactin® column is inactive.	Check the column activity by applying 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. 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.
The column is not properly regenerated.	Increase the volume of 100 mM NaOH and regenerate again. Efficient regeneration can be visualized by the addition of HABA. When HABA is added to the column, it changes its color from yellow to red.
Flow rate is too fast.	Reduced flow rates may increase yields depending on the given recombinant protein.
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	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% Tergitol, 2% Tween 20, etc.).
Contaminants are biotinylated proteins.	Add avidin or BioLock, a biotin blocking solution containing avidin.