Protocol

For research use only

Protein purification with Strep-Tactin® 4Flow® high capacity resin

Affinity-based protein purification with gravity flow columns

1 DESCRIPTION

Strep-Tactin® 4Flow® high capacity can be used for gravity flow purification and consists of a 4 % agarose coated with Strep-Tactin®. The low concentrated 4Flow® agarose achieves higher yields for large proteins compared to a 6 % agarose. Strep-Tactin® specifically interacts with the Strep-tag®II as well as the Twin-Strep-tag® via the engineered biotin binding pocket and 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®, unspecific binding can be avoided.

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 can be regenerated with 100 mM NaOH.

2 GENERAL INFORMATION AND REQUIRED MATERIAL

2.1 Available formats

The following protocol is intended for gravity flow column-based protein purification with Strep-Tactin® 4Flow® high capacity. For customer specific gravity flow columns, the resin is offered as 50% suspension. Prepacked gravity flow columns containing Strep-Tactin® 4Flow® high capacity resin with 0.2 ml, 1 ml, 5 ml or 10 ml column bed volume are offered by IBA Lifesciences.

To allow an efficient purification with Strep-Tactin® we recommend using column purification instead of batch applications. It is crucial that protein binding takes place on the column. Even 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. If batch purification is attempted, the use of MagStrep® Strep-Tactin®XT beads is recommended.

2.2 Recommended Buffers

All necessary buffers for protein purification and subsequent regeneration of the resin are listed in the following table. IBA Lifesciences provides Buffer W and Buffer E 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® and Strep-Tactin®XT 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 resins. Always use freshly prepared NaOH.

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.html. Please note that the pH value of the buffer should be between 7-8.

2.3 Large sample volumes

If the target protein should be purified from larger sample volumes, protein may leach during the sample application and washing steps. In these cases, we recommend the use of StrepTactin®XT 4Flow® and StrepTactin®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.

We recommend the use of the WET FRED application aid. It 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.

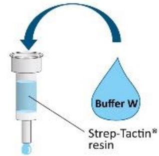
2.4 Biotin in your sample

Eukaryotic cultivation media (for mammalian, insect and yeast cell cultures) may contain significant amounts of biotin. Please note that 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 and prevents binding of the Strep-tag®II and Twin-Strep-tag® fusion protein and does not allow regeneration of the Strep-Tactin® 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 avidin or BioLock 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 biotinylated proteins is provided at https://www.iba-lifesciences.com/download-area.html.

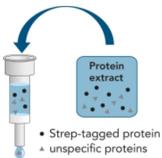
3 PROTOCOL

3.1 Gravity flow column-based protein purification

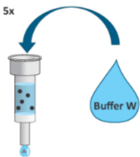
It is recommended to perform protein purification at 2-8 °C. However, if protein purification has to be performed at room temperature and columns are transferred from cold to room temperature air bubbles may form due to restricted solubility of air at elevated temperatures. Therefore, it is recommended to equilibrate the columns immediately after exposure to higher temperatures with buffer that is equilibrated at such temperatures.



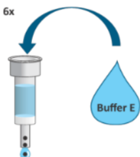
3.1.1 Remove the top cap from the column first, then twist off the lower cap. If the caps are removed in reverse order, air may enter the column bed. Let the storage buffer flow through. The column cannot run dry under gravity flow. Equilibrate the Strep-Tactin® column with 2x 1 CV (column bed volume) 1x Buffer W. The column bed volume corresponds to the amount of resin in the gravity flow column.



3.1.2 Centrifuge the sample (18,000 x g, 5 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. The sample volume should be in the range of 0.5 and 10 CVs. Concentrated samples are preferred, since large sample volumes with the target protein in low concentrations may lead to reduced yields. Collect the flow through for subsequent SDS-PAGE analysis.



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



3.1.4 Add 6 x 0.5 CV 1x Buffer E and collect the eluate in 0.5 CV fractions. The main protein content should be in the 2nd to 5th fraction.

3.1.5 Analyze protein purification results by SDS-PAGE.

3.2 Regeneration and storage of the gravity flow column

3.2.1 For regeneration, freshly prepare 100 mM NaOH.

Apply 15 CV 100 mM NaOH to the column.

3.2.2 After the NaOH has run through the column, use at least 15 CV 1x Buffer W to remove NaOH.

3.2.3 Overlay the column with 2 CV 1x Buffer W. Store the FPLC column 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® column

pH is not correct.	The pH should between pH 7.0 and pH 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® column is inactive.	Check activity with HABA. To avoid inactivation of the column due to biotin/biotinylated proteins, add avidin (or BioLock) to the sample, 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 column. Even a pre incubation of resin and lysate prior to filling the resin into a 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.
Column has not been fully regenerated.	To 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.

4.2 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.	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 BioLock or avidin. Check section 2.4 for more details.

4.3 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 to perform protein purification 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.

Check our Downloads page

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

for the latest version of this manual.

Info on warranty / licensing and trademarks available at:

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

If you have any questions, please contact

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We are here to help!

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