Protein Production & Assays

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Protocol

Protein purification with Strep-Tactin® resins

Affinity-based protein purification with gravity flow columns

1 DESCRIPTION

Strep-Tactin® Sepharose®, Superflow® and MacroPrep® as well as Strep-Tactin® Superflow® high capacity can be used for gravity flow purification. 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® resins 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.

2 GENERAL INFORMATION AND REQUIRED MATERIAL

The following protocol is intended for gravity flow column-based protein purification with Strep-Tactin® Sepharose®, Superflow® and MacroPrep® or Strep-Tactin® Superflow® high capacity. For customer specific gravity flow columns, all Strep-Tactin® resins are offered as 50% suspension. Prepacked gravity flow columns containing Strep-Tactin® Sepharose® and Strep-Tactin® Superflow® high capacity 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 a 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 MagStrep "type 3" XT beads is recommended.

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® 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.
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.

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®		
0.2 ml	0.1-2 ml	0.1-20 ml	5 x 0.2 ml	6 x 0.1 ml
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
10 ml	5-100 ml	5-1000 ml	5 x 10 ml	6 x 5 ml

*Adjust sample volume according to binding capacity of the column (please refer to the appropriate data sheet) 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.

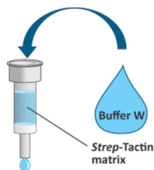
The WET FRED enables convenient application of large sample volumes, e.g., cell culture supernatant, to a gravity flow column in a simple way (for 1 ml gravity flow columns Cat. No. 2-0911-001; for 5-10 ml gravity flow columns Cat. No. 2-9010-001) and is re-usable.

Eukaryotic cultivation media (for mammalian or insect cell expression as well as for yeast) 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-protein.html.

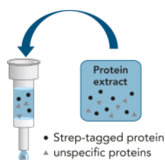
3 PROTOCOL

3.1 Gravity flow column-based protein purification

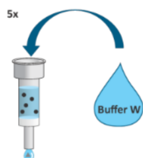
It is recommended to perform protein purification at 2-8 °C. However, if protein purification has to be performed at room temperature and columns are transferred from cold to room temperature air bubbles may form due to restricted solubility of air at elevated temperatures. Therefore, it is recommended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



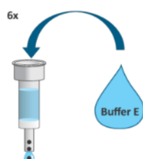
3.1.1 Remove the top cap from the column first, then twist off the lower cap. If the caps are removed in reverse order, air may enter the column bed. Let the storage buffer flow through. The column cannot run dry under gravity flow. Equilibrate the Strep-Tactin® column with 2x 1 CV (column bed volume) 1x Buffer W. The column bed volume corresponds to the amount of resin in the gravity flow column. This means that a 0.2 ml gravity flow column contains a column bed volume of 0.2 and therefore 0.4 ml 1x Buffer W has to be applied.



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.

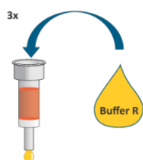


3.1.4 Add 6 x 0.5 CV 1x Buffer E and collect the eluate in 0.5 CV fractions. The main protein content should be in the 2nd to 5th fraction.

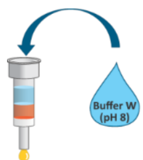
3.1.5 Analyze protein purification results by SDS-PAGE.

3.2 Regeneration and storage of the gravity flow column

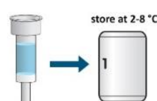
A video of the color-based functionality check using a Strep-Tactin® Sepharose® gravity flow column as an example is available at https://www.youtube.com/watch?v=N__1Y8b9s8c



3.2.1 Wash the column 3 times with 5x 1 CV 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. Regeneration is complete when the red color on the bottom of the column has the same intensity as on top of the column. If this is not the case, use more 1x Buffer R.



3.2.2 Overlay the column with 2 ml 1x Buffer W or 1x Buffer R.



3.2.3 Close the column with the top cap and then with the lower cap. Store the column at 2-8 °C. Remove 1x Buffer R by washing with 2x 4 CV of 1x Buffer W prior to the next use.
Exception: In case of Strep-Tactin® Superflow® high capacity, use 4 CV 1x Buffer W at pH 10.5 for HABA removal. Immediately afterwards, exchange the column buffer to 1x Buffer W pH 8.0 as long-term exposure to pH 10.5 may be detrimental to the resin.

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 8.5.
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.

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% Triton X-100, 2% Tween 20, 0.1% CHAPS, etc.).
Contaminants are biotinylated proteins.	Add biotin blocking solution, BioLock, or avidin.

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

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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