# Purification of biotinylated proteins with Strep-Tactin®XT resin

Efficient purification of in vivo biotinylated Avi-tag proteins using gravity flow columns

## 1. Description

Strep-Tactin®XT 4Flow® high capacity consists of a 4% agarose coupled with streptavidin variant Strep-Tactin®XT. Strep-Tactin®XT was developed for purification of Strep-tag®II and Twin-Strep-tag® fusion proteins. However, due to its streptavidin origin, Strep-Tactin®XT still has an affinity for biotin (µM) and is therefore suitable for purifying biotinylated proteins as well. The elution occurs under mild conditions with a specific competitor, biotin, which releases the biotinylated target protein from the engineered binding pocket without influencing the target protein's properties. If necessary, biotin can be easily removed via dialysis, size exclusion chromatography, or cross flow ultrafiltration after purification.

## 2. Required material and recommendations

The protocol is intended for gravity flow column-based purification of in vivo biotinylated proteins with Strep-Tactin®XT 4Flow® high capacity. Prepacked gravity flow columns are offered by Cube Biotech. For customer specific gravity flow columns, Strep-Tactin®XT 4Flow® high capacity is also available as 50% suspension. To allow an efficient purification with Strep-Tactin®XT, we recommend using column purification instead of batch applications. For batch purifications, we recommend the application of MagStrep® Strep-Tactin®XT beads.

All necessary buffers for protein purification and subsequent regeneration of the resin are listed in the following table.

| Buffers/solutions | | Cat. No. | Quantity |
|---|---|---|---|
| Wash buffer | Buffer W (10 x)<br>(1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, pH 8) | 2-1003-100 | 100 ml |
| Elution buffer | Buffer BXT (10 x)<br>(1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, 500 mM biotin, pH 8) | 2-1042-025 | 25 ml |
| Regeneration buffer | 100 mM NaOH (working concentration) | Not provided.<br>Prepare freshly. | - |

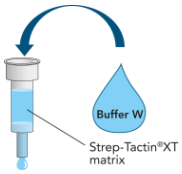
Please note that the wash & elution buffers are provided as 10-fold concentrated solutions. Prior to application, mix one part of 10-fold concentrated buffer with nine parts of deionized water.
The composition of all purification buffers can be modified to suit the properties of the target protein. A list with compatible reagents is available at https://www.iba-lifesciences.com/download-area-protein.html. Please note that the resin is stable at pH 4-10.

If the target protein should be purified from larger sample volumes, we recommend the application of the WET FRED – an application aid for Strep-Tactin® and Strep-Tactin®XT gravity flow columns (for 1 ml gravity flow columns Cat. No. 2-0911-001; for 5-10 ml gravity flow columns Cat. No. 2-9010-001).

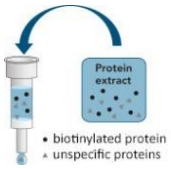
# 3. Protocol

## 3.1. Gravity flow column-based protein purification

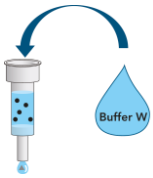
The concentration of free biotin should be < 10 uM (< 2,500 ug/l). Higher concentrations can affect the protein purification efficiency of biotinylated proteins.




**3.1.1.** It is recommended to perform protein purification at **2-8 °C**.
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®XT 4Flow® high capacity 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.

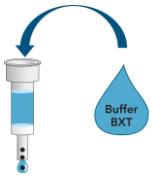



**3.1.2.** Centrifuge the sample **(40,000 x g, 15 min, 4 °C)** to remove any aggregates that may have formed. Add the sample to the column and let it completely flow through by gravity flow.
*Optional:* Collect the flow through for subsequent SDS-PAGE analysis or for a second purification round to improve protein yield.




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

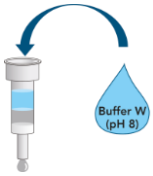
Standard elution is performed using 50 mM biotin (1x Buffer BXT). Depending on protein, effective elution is also possible using 5-10 mM biotin.



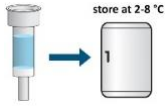

**3.1.4**. Add **6x 0.5 CV 1x Buffer BXT** and collect the eluate in 0.5 CV fractions. Optional: To obtain most of the eluted protein in one fraction with a high concentration add 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2), and finally 0.8 CV (E3). The main protein content should be in E2. Analyze protein purification results by SDS-PAGE.

## 3.2. Regeneration and storage of the gravity flow column


**3.2.1.** Wash the column with 15 CV of **100 mM freshly prepared NaOH.**




**3.2.2.** Immediately wash the column by adding **8 CV 1x Buffer W.**




**3.2.3.** Overlay the column with **2 ml 1x Buffer W**. Close the column with the top cap and then with the lower cap. Store the column at **2-8 °C**.
**Optional:** Storage in 20% Ethanol is possible for 12 months without loss of performance.

# 4. Troubleshooting

| **No or weak binding to Strep-Tactin®XT column** | |
|---|---|
| pH is not correct | The resin is stable at pH 4-10. |
| Biotinylation is not present, or protein has been degraded. | 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.<br>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. |
| Low yields/expression levels | When working with cell lysate, reduce the amount of lysis buffer for cell pellet resuspension to increase the concentration of your protein of interest. |
| Strep-Tactin®XT column is inactive. | 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.<br>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. |
| **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 | When working with *E. coli*, use protease deficient expression strains.<br>Add protease inhibitors before/after cell lysis. Fuse tag 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. |
| Contaminants are covalently linked to the recombinant protein via disulfide bonds. | Add reducing reagents to all buffers necessary for cell lysis and protein purification. |

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|---|---|
| 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.). |
| Elution contains other biotinylated proteins. | Secondary purification by size exclusion or ion exchange chromatography might be necessary. |

| **Air bubbles in the column** |
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| 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 performing chromatography 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. |