Strep-Tactin®XT Purification

IBA's 3rd generation Strep-tag® system is based on the novel **Strep-Tactin®XT** in combination with the **Twin-Strep-tag®**.
Strep-Tactin®XT has a binding affinity in low pM ranges for the Twin-Strep-tag® and in nM ranges for the Strep-tag®II, still maintaining binding reversibility, mild recovery of immobilized target proteins and straightforward regeneration of the resin for re-use. The improved binding affinity of Strep-Tactin®XT ensures highest protein purities (> 95 %) under physiological conditions combined with sharp elution profiles for highly concentrated proteins.

Further, Strep-Tactin®XT now enables new applications: high throughput screening, batch purification, purification using denaturing conditions, additives and detergents as well as protein interaction studies. This versatility makes the system superior to all other available tag based affinity purification systems.

The main differences between the Strep-Tactin® and Strep-Tactin®XT purification procedure:

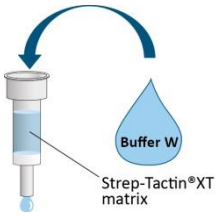
- Strep-Tactin®XT requires elution of the target protein with Buffer BXT containing **50 mM Biotin**
  (Due to the high binding affinity desthiobiotin cannot be used for elution.)

- Regeneration of the Strep-Tactin®XT Superflow® resin with **10 mM NaOH**
  (Since biotin is used for elution of the target protein, the column needs to be recovered with NaOH instead of HABA. However, HABA can be used to monitor a successful regeneration of the column subsequent to NaOH treatment.)

Short Protocol of the Strep-Tactin®XT chromatography cycle

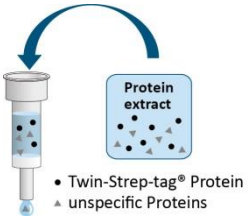
Perform all operations at a temperature amenable to the stability of your recombinant protein (between 4°C and 25°C). To achieve optimal purification results, comply with the specified volumes and their ratios (column bed, washing volumes etc., see page 3). At low expression levels, increase cell extract volumes to take advantage of the column capacity, without changing other volumes.

Strep-Tactin®XT Purification
-Short Protocol-



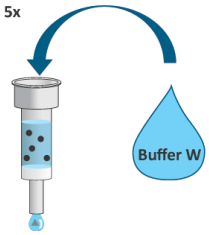
Equilibrate the column

1. Remove top cap from column first, then twist off lower cap. Remove storage buffer and **equilibrate column with 2 CV (column bed volume) Buffer W** (100 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA)



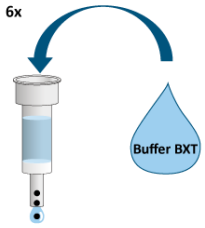
Apply the protein extract

2. Frozen cell extracts have to be centrifuged prior to application (18.000 x g, 5 min, 4°C) in order to remove any aggregates that may have formed. **Apply the cleared extract to the column.**



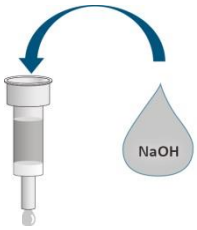
Wash the column

3. **Wash column 5x with 1 CV of Buffer W**
   Collect the wash fractions (1 CV each) and optionally save 2 µl of each subsequent wash fraction for application on an analytical SDS-PAGE.



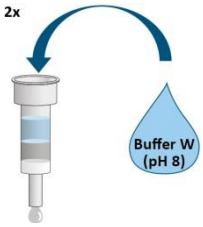
Elute protein

4. **Add 6x 0.5 CV of Buffer BXT**
   Collect the eluate in 0.5 CV fractions.
   Option: To get high protein concentrations in one fraction add 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2) and finally 0.8 CV (E3). Main protein content should be in E2. 20 µl samples of each fraction can be used for SDS-PAGE analysis.



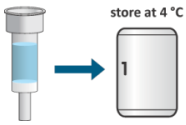
Regenerate column

5. **Wash the column with 4 CV of 10 mM NaOH**
   Always use freshly prepared 10 mM NaOH.
   Strep-Tactin®XT Superflow® resin cannot be regenerated using HABA (Buffer R). However, after treatment with NaOH, operability can be confirmed by application of Buffer R which induces an orange-shift in case of a successful regeneration.



Remove NaOH and equilibrate

6. **Immediately remove NaOH by adding 8 CV Buffer W (pH 8.0).**



Store column

7. **Column can be stored in Buffer W at 4°C.**

Recommended volumes for working with Strep-Tactin®XT columns

Column bed volume (CV)	Wash buffer volume	Elution buffer volume
0.2 ml	5 x 0.2 ml	6 x 0.1 ml
1 ml	5 x 1 ml	6 x 0.5 ml
5 ml	5 x 5 ml	6 x 2.5 ml
10 ml	5 x 10 ml	6 x 5 ml

Table 1: Recommended buffer volumes for chromatography on Strep-Tactin®XT columns
Adjust protein extract volume according to binding capacity of the column (please refer to the appropriate data sheet) and apply the extract as concentrated as possible in the recommended volume range. Note that these volumes are average values which can be different for certain proteins.

Buffer composition:

Buffer W	100 mM Tris/HCl, pH 8.0; 150 mM NaCl; 1 mM EDTA
Buffer BXT	100 mM Tris/HCl, pH 8.0; 150 mM NaCl; 1 mM EDTA; 50 mM biotin
Buffer R (optional)	100 mM Tris/HCl, pH 8.0; 150 mM NaCl; 1 mM EDTA; 1 mM HABA

Biotin in cell culture media

Please note that biotin binds with high affinity to Strep-Tactin®XT, thereby it efficiently precludes binding of Twin-Strep-tag®.
Especially culture media for mammalian cell or insect cell cultivation may contain significant amounts of biotin. Thus, if recombinant proteins are secreted to the culture medium, biotin must be masked by the addition of avidin or BioLock prior to Strep-Tactin®XT chromatography. Alternatively, biotin can be removed by dialysis or gel filtration.
More information, particularly a list enumerating the biotin content of different cell culture media, can be found here: http://www.iba-lifesciences.com/download-area.html.

For a more detailed protocol and troubleshooting please download the comprehensive Strep-Tactin®XT Purification Manual from http://www.iba-lifesciences.com/download-area.html.

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