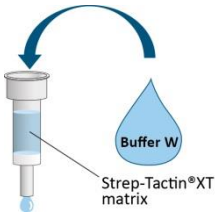
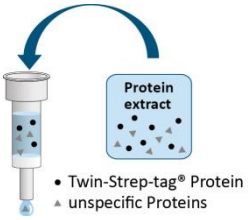
Strep-Tactin®XT Purification
-Short Protocol-



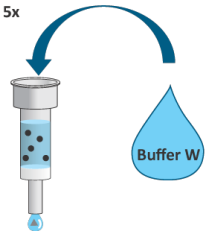
Equilibrate the column

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)



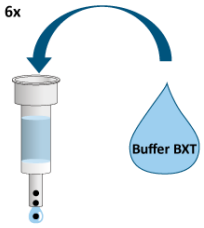
Apply the protein extract

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.**



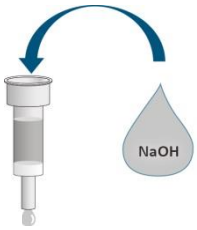
Wash the column

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.



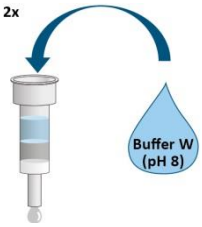
Elute protein

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.



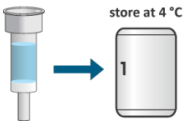
Regenerate column

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 two times 4 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 (Cat.No. 2-0204-015) or BioLock (Cat.No. 2-0205-050) 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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