Strep-Tactin®XT Purification

Strep-Tactin®XT is an engineered streptavidin with four biotin binding pockets. As a result, it is able to bind recombinant proteins tagged with Strep-tag®II or Twin-Strep-tag®. The affinity of Strep-Tactin®XT for Strep-tag®II is in the nM range and increases for the Twin-Strep-tag® up to the pM range. Immobilized proteins are eluted under mild conditions by an excess of the competitor biotin. The buffer composition can also be adapted specifically to each target protein. This means that there are no restrictions in the application for certain protein classes, such as metal ion containing proteins, membrane proteins or pH-sensitive proteins. After purification, the resin can be regenerated with Buffer XT-R or alternatively with fresh NaOH solution (10 mM) and reused three to five times without loss of yield.
Further, Strep-Tactin®XT can be used for immobilization and detection in analytic applications like ELISA or SPR – or you can develop your own assay. This versatility makes the Strep-tag technology superior to all other available tag based affinity purification systems.

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

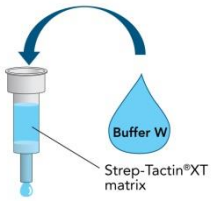
- Strep-Tactin®XT requires elution of the target protein with Buffer BXT containing 50 mM biotin. Due to the lower affinity of Strep-Tactin®XT for desthiobiotin, the latter cannot be applied for elution.

- For the regeneration of the Strep-Tactin®XT resins Buffer XT-R has to be used. Since biotin is required for elution of target proteins, the resin needs to be recovered with Buffer XT-R (3 M $MgCl_2$) instead of Buffer R (HABA). However, Buffer R can be applied to monitor a successful regeneration of the resin subsequent to Buffer XT-R treatment.

Short Protocol of the Strep-Tactin®XT chromatography cycle

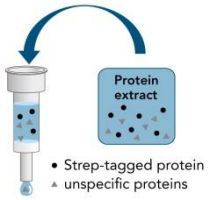
Perform all operations at a temperature suited to the stability of your recombinant protein (between 4°C and 25°C). To achieve optimal purification results, comply with the specified volumes and their ratios (see page 3). At low expression levels, increase cell extract volumes to take advantage of the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

Strep-Tactin®XT Purification - Short Protocol



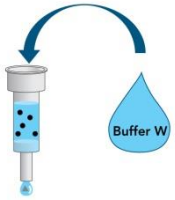
Equilibrate column

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



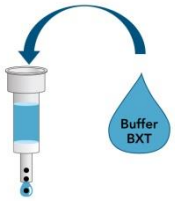
Apply protein extract

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.**



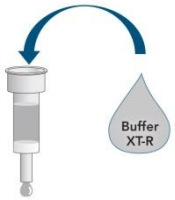
Wash column

3. **Wash column with 5 x 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 gel.



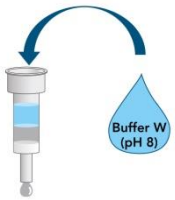
Elute protein

4. **Add 6 x 0.5 CV of Buffer BXT.**
Collect the eluate in 0.5 CV fractions. Optional: 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.



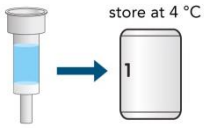
Regenerate column

5. **Wash column with 6 CV of Buffer XT-R.**
Strep-Tactin®XT resin cannot be regenerated using Buffer R (HABA). However, after treatment with Buffer XT-R, operability can be confirmed by application of Buffer R which induces an orange-shift in case of a successful regeneration. Instead of Buffer XT-R, freshly prepared 10 mM NaOH can also be used.



Remove Buffer XT-R and equilibrate

6. **Immediately remove Buffer XT-R by adding 2 x 4 CV Buffer W (pH 8.0).**



Store column

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

Table 1: Calculation of the required buffer volumes based on the column bed volume. The column bed volume corresponds to the amount of resin. This means a 0.2 ml gravity flow column contains a column bed volume of 0.2. Example: 6 x 1 CV buffer should be applied to a 0.2 ml column. In this case, 6 x 0.2 ml or a total of 1.2 ml buffer must be applied to the column.

n x CV		column bed volume (CV)				required buffer volume (ml)
		0.2	1	5	10	
quantity (n)	0.5	0.1	0.5	2.5	5	
	0.6	0.12	0.6	3	6	
	0.8	0.16	0.8	4	8	
	1	0.2	1	5	10	
	1.6	0.32	1.6	8	16	
	2	0.4	2	10	20	
	5	1	5	25	50	
	6	1.2	6	30	60	
	8	1.6	8	40	80	
	15	7.5	15	75	150	

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 XT-R	3 M $MgCl_2$
Buffer R (optional)	100 mM Tris/HCl, pH 8.0; 150 mM NaCl; 1 mM EDTA; 1 mM HABA

Biotin in cell culture media

Especially culture media for mammalian or insect cell cultivation may contain significant amounts of biotin. In case of Strep-Tactin®XT implementation for protein purification, biotin does not have to be masked by the addition of avidin or biotin blocking solution (BioLock). Compared to Strep-Tactin®, Strep-Tactin®XT has a lower affinity for biotin. Therefore, the binding of the Strep-tag®II or Twin-Strep-tag® is not disturbed by biotin contained in culture media. However, in order to avoid co-purification of biotinylated proteins, BioLock (cat. no. 2-0205-050) has to be added.

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

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