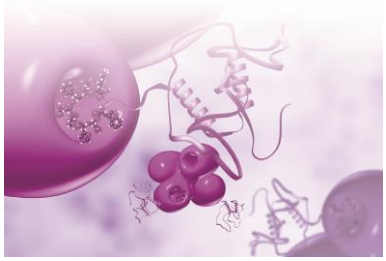
Solutions For Life Sciences

Application Note



Strep-Tactin®XT beats Strep-Tactin® -

Benefits of the 3rd generation Strep-tag® system

Introduction

The usage of protein tags in combination with affinity chromatography has significantly eased the purification of recombinant proteins. The plethora of available tags, each harboring distinct advantages and disadvantages, offers a wide range of fields of applications, like purification, detection and immobilization. However, the usage of solely one tag for all of the aforementioned applications is rather unlikely due to the tag-specific binding parameters. Therefore, a single tag which could be used for nearly all applications would be highly attractive. IBA's 3rd generation Strep-Tactin®XT:Twin-Strep-tag® affinity purification system is the most versatile purification system on the market with respect to its applicability.

Initially developed for the specific reversible binding to the biotin pocket of streptavidin, Strep-tag®II depicted the start of the Strep-tag® system[1,2]. Streptavidin was later on engineered to obtain the highly selective Strep-Tactin®, which binds the Strep-tag®II with an affinity of about 1 µM which is nearly 100-times higher compared to streptavidin. The competitive elution is performed with desthiobiotin, an inexpensive, reversibly binding and stable analogue of biotin. The further improved Twin-Strep-tag® is a sequential arrangement of two Strep-tag®II sequences (total size of 28 aa). This tag enables the same mild and rapid purification as Strep-tag®II but, in addition, has an increased affinity for Strep-Tactin® (low nM range). Further, the Twin-Strep-tag® allows efficient purification even in batch or of diluted proteins e.g. directly from cell culture supernatants[3].

The recently developed Strep-Tactin®XT (**x**tra **t**ight) now expands the 3rd generation of the Strep-tag® system representing a breakthrough in protein science (**Fig 1.**). Strep-Tactin®XT has a binding affinity in low pM ranges for Twin-Strep-tag® still maintaining binding reversibility and mild recovery of immobilized proteins. Hence, Strep-Tactin®XT not only enables highest protein purities under physiological conditions and sharp elution profiles for highly concentrated proteins, but also offers new applications in the field of high throughput screening, purification using denaturing conditions and assay development or protein interaction studies by Biacore.

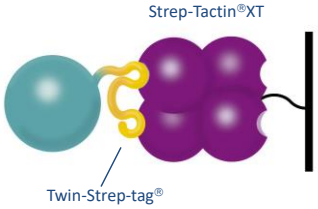


Fig 1. Schematic of the Strep-Tactin®XT:Twin-Strep-tag® interaction.

This application note discusses the purification of several proteins either being purified using Strep-Tactin® Superflow® or Strep-Tactin®XT Superflow® resins. During analysis, major emphasis was devoted to the purification efficiency and the resulting overall protein yield.

Material and Methods

In case of prokaryotic expression, the respective genes of interest were cloned into an appropriate IBA expression vector and were used for transformation of *E. coli* BL21 cells. Cells were grown in LB or HD medium containing ampicillin and were induced at an appropriate optical density. Subsequently, the cells were harvested and resuspended in Buffer W (100 mM Tris/HCl, pH 8.0; 150 mM NaCl; 1 mM EDTA). Cell disruption was performed using ultra-sonication followed by removal of the cell debris by centrifugation. The resulting supernatant (2 ml for GFP and CP; and 3 ml for GAPDH and mCherry) was applied on a 1 ml Strep-Tactin® Superflow® or Strep-Tactin®XT Superflow® column, respectively. Washing steps, each with 1 column volume (CV) of Buffer W, were carried out until absorption at 280 nm reached a constant level. For Strep-Tactin® resin elution of the target proteins was carried out by applying 0.8 CV, 1.4 CV and 0.8 CV of Buffer W further containing 2.5 mM desthiobiotin (Buffer E). In contrast, elution from Strep-Tactin®XT was carried out with Buffer BXT (Buffer W + 50 mM biotin) using the same elution profile. Fractions were monitored by UV-Vis at 260 nm and 280 nm (Nanodrop2000, Thermo Fisher) and were then further analyzed by SDS-PAGE.

For expression in mammalian cells, the gene encoding a customer protein was cloned into IBA expression vector pDSG-IBA102 and used for transfection of MEXi-293E cells. Cells were transfected in MEXi-TM medium according to the MEXi instruction manual. 4 hours post transfection the culture was diluted with one volume of MEXi-CM medium. Cells were grown until a viability of 75 %. Afterwards, cells were removed by centrifugation and the resulting supernatant was further processed according to the MEXi instruction manual. Briefly, 10x Buffer W and BioLock Biotin Blocking solution were added to the supernatant. Subsequently, 10 ml cell culture supernatant were applied on a 1 ml Strep-Tactin® and Strep-Tactin®XT column, respectively. The columns were washed with 5 CV Buffer W. The following elution was carried out by applying 0.8, 1.4 and 0.8 CV of Buffer E (for Strep-Tactin®) or Buffer BXT (for Strep-Tactin®XT).

Results

High binding affinity of Strep-Tactin®XT allows intensive washing steps

Strep-Tactin® and Strep-Tactin®XT resins were compared with respect to their performance when intense washing steps were performed during purification. After application of the cleared lysate / culture supernatant both columns were washed with Buffer W until a constant absorbance at 280 nm was reached followed by subsequent elution of the target protein. After five washing steps (5 CV) a high A260/A280 nm was still observed in some samples indicating DNA impurities. With increasing number of washing steps these impurities were significantly reduced for both columns yielding in an almost complete loss in absorption after 8 CV for the Strep-Tactin®XT resin. On the contrary, a constant absorption signal could still be observed for Strep-Tactin®. SDS-PAGE analysis revealed an abortive elution of the target proteins during these washing steps which finally led to a reduced overall protein yield (**Fig. 2**).

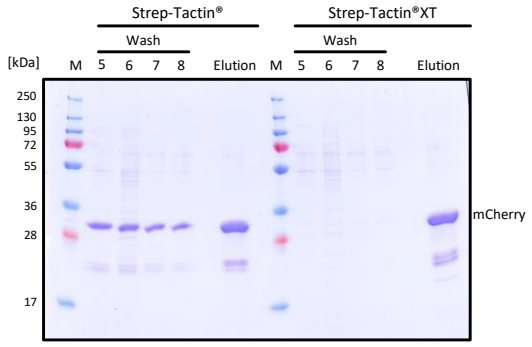


Fig 2. Enhanced binding of Twin-Strep-tag®-mCherry to Strep-Tactin®XT compared to Strep-Tactin®. A significant loss of mCherry occurred during the washing steps if Strep-Tactin® is used for purification. In the case of Strep-Tactin®XT no target protein is lost during the purification procedure. Western blot analysis revealed that remaining impurities were degradation products of mCherry.

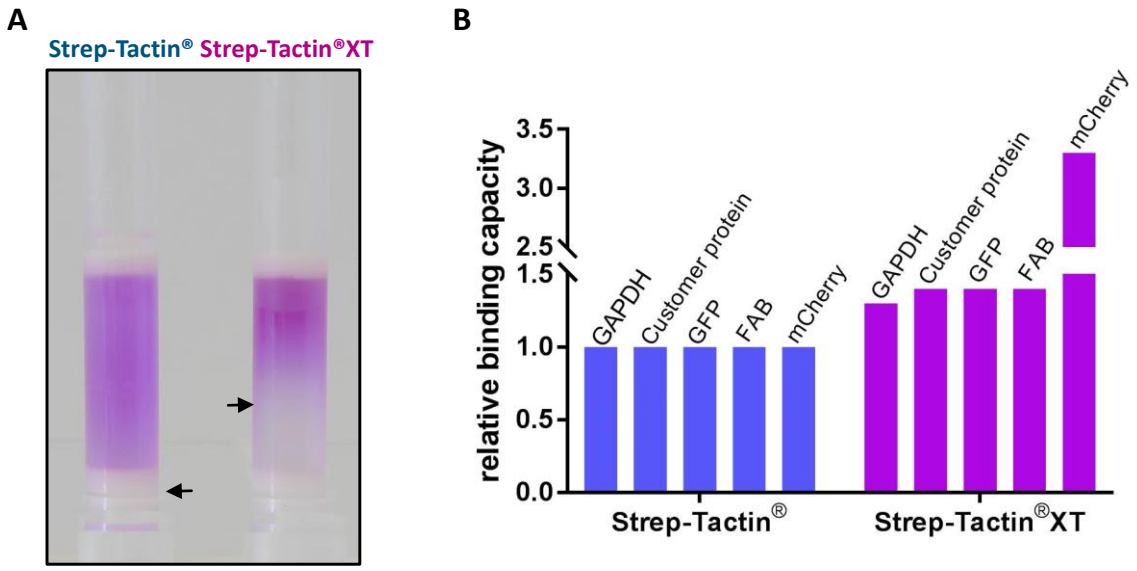


Fig. 3. Strep-Tactin®XT provides higher protein yields than Strep-Tactin®. **A** Relative binding capacity of Strep-Tactin® and Strep-Tactin®XT visualized by binding of Twin-Strep-tag®-mCherry protein. The same volume of protein extract derived from the same batch was loaded on both columns followed by washing with Buffer W. mCherry protein harbors a peak absorbance at 587 nm leading to its typical purple-red color. **B** Comparison of the relative binding capacity of numerous proteins. On average, Strep-Tactin®XT provides at least 1.5-fold more protein than Strep-Tactin®.

Strep-Tactin®XT provides higher protein yields

In a second series of experiments both resins were compared with respect to their binding capacity and total protein yield. The relative binding capacity was visualized by monitoring the binding of Twin-Strep-tag®-mCherry fluorophore to both resins (**Fig. 3A**). After application of a defined volume of protein extract on a 1 ml Strep-Tactin® Superflow® column the maximum resin binding capacity for mCherry was almost reached. In contrast, when the equal amount was applied on a Strep-Tactin®XT Superflow® column only one-third of the resin was occupied with the target protein. Thus, Strep-Tactin®XT ensures sharp elution profiles for highly concentrated protein. In addition, for all proteins tested Strep-Tactin®XT led to higher protein yields compared to Strep-Tactin®. On average, at least 1.5-fold more protein could be eluted from this resin (**Fig. 3B**).

Conclusions

Both resins were successfully used for the purification of Twin-Strep-tag® fusion proteins from bacterial and mammalian sources. However, Strep-Tactin®XT provides clear benefits compared to Strep-Tactin® thus leading to an unparalleled performance. Due to the high binding affinity of the Strep-Tactin®XT:Twin-Strep-tag® pair bound proteins can be washed intensively without the unnecessary loss of target. Further, IBA's 3rd generation Strep-tag® system allows the purification of higher amounts of target proteins leading to a more convenient and effective purification procedure.

References

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2. Voss S, Skerra A (1997) Mutagenesis of a flexible loop in streptavidin leads to higher affinity for the Strep-tag II peptide and improved performance in recombinant protein purification. Protein Eng 10:975–982.

3. Schmidt TGM, Batz L, Bonet L, Carl U, Holzapfel G, Kiem K, Matulewicz K, Niermeier D, Schuchardt I, Stanar K (2013) Development of the Twin-Strep-tag® and its application for purification of recombinant proteins from cell culture supernatants. Prot Expr Purif 92:54-61.