iba Solutions For Life Sciences

Protein Production & Assays

Application Note

STREP-TACTIN®XT:TWIN-STREP-TAG®

Advantages compared to the His-tag purification system

Introduction

The usage of protein tags in combination with affinity chromatography has significantly relieved the purification of recombinant proteins from a multitude of different hosts. The plethora of available tags made protein purification, which had been tedious work during the last decades, a "press button"-method. However, there are still pitfalls to overcome: For instance, high-level expression of many recombinant proteins in *Escherichia coli* often leads to the formation of highly aggregated protein commonly referred to as inclusion bodies[1]. A purification of such proteins is in most of the cases only possible under denaturing conditions using high concentrations of either urea or guanidinium hydrochloride.

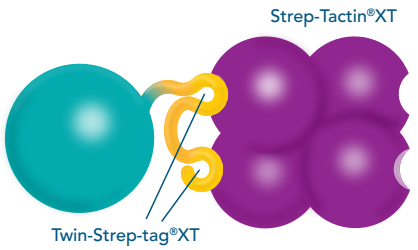

Fig 1. Schematic of the Strep-Tactin®XT:Twin-Strep-tag® interaction.

To date the highly selective Strep-Tactin® has not been well-suited to ensure an efficient purification under denaturing conditions. The 3rd generation of the Strep-tag® system represents a breakthrough in protein science also allowing the highly effective purification of proteins under denaturing conditions. The recently developed Strep-Tactin®XT (**x**tra **t**ight) has a binding affinity in low pM ranges for the Twin-Strep-tag® still maintaining the binding reversibility and the mild recovery of immobilized proteins. Hence, Strep-Tactin®XT enables highest protein purities under physiological conditions, sharp elution profiles for highly concentrated proteins and regeneration of the resin for reuse.

This application note discusses the benefits of the Strep-Tactin®XT:Twin-Strep-tag® purification system compared to the His-tag system using both native and denaturing conditions (Fig 1.). Therefore, we directly compared the purity of target proteins after being purified either under native conditions and after being purified in the presence of up to 6 M urea (denaturing conditions).

Material and Methods

The genes encoding for mCherry, GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and a customer protein (CP) were cloned into an IBA expression vector leading to Twin-Strep-tag® and His_6-tag fusion proteins which were then used for transformation of *E. coli* BL21 cells, respectively. The expression and subsequent cell harvest were conducted according to IBA's protocol PR86-0001. Briefly, 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 the appropriate Buffer. For His_6-tag proteins Ni-NTA Lysis Buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl 10 mM imidazole) and for Twin-Strep-tag® proteins Buffer W (100 mM Tris/HCl, pH 8.0, 150 mM NaCl; 1 mM EDTA) was used. Cell disruption was performed using ultrasonication followed by removal of the cell debris by centrifugation. mCherry and GAPDH were purified under native conditions whereas the customer protein was purified under denaturing conditions.

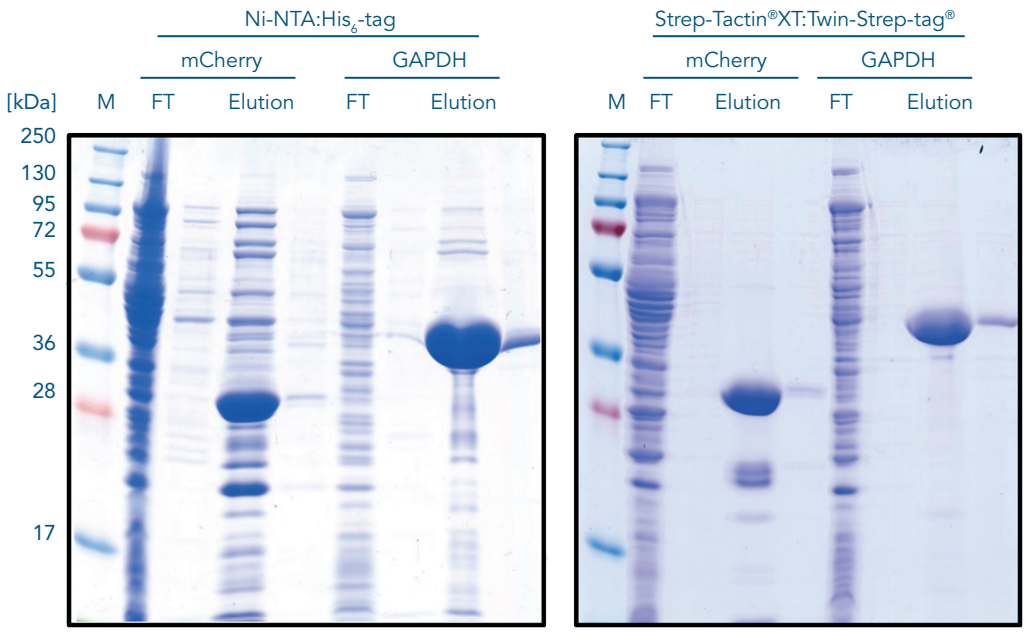

Fig 2. SDS-PAGE analysis of the purification of mCherry and GAPDH under native conditions. (left) Ni-NTA:His_6-tag system (right) Strep-Tactin®XT:Twin-Strep-tag® system. According to Western blot analysis the residual impurities in case of the mCherry purification with Strep-Tactin®XT are co-purified degradation products.

For purification under native conditions 3 mL of each of the resulting supernatant was applied on a 1 ml Ni-NTA, a Strep-Tactin® and a Strep-Tactin®XT Superflow® gravity flow column equilibrated with either Ni-NTA Lysis Buffer or Buffer W. The Ni-NTA column was washed 4 times with 2 column volumes (CV) Ni-NTA Wash Buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl 20 mM imidazole). Both Strep-Tactin® columns were washed 8 times with 1 CV Buffer W. Elution was performed by using 6 times 0.5 CV of the appropriate elution Buffer (Ni-NTA: 50 mM NaH_2PO_4 pH 8.0 300 mM NaCl 250 mM imidazole; Strep-Tactin®: Buffer E (Buffer W + 2.5 mM desthiobiotin) and Strep-Tactin®XT: Buffer BXT (Buffer W + 50 mM biotin)).

For purification of the customer protein under denaturing conditions the cell pellet was dissolved at room temperature under constant stirring for 15-60 min in Buffer W containing 8 M urea (Twin-Strep-tag®) or Buffer B (100 mM NaH_2PO_4 pH 8.0, 10 mM Tris/HCl 8 M urea; His_6-tag) followed by further centrifugation step. Prior to application on the column the solubilized protein was adjusted to 4 M and 6 M urea by dilution with Buffer W for Strep-Tactin® and Strep-Tactin®XT purification, respectively. Afterwards, the supernatants were applied on a 1 ml Ni-NTA, a Strep-Tactin® and a Strep-Tactin®XT column equilibrated in the appropriate washing buffer further containing the respective urea concentration. The columns were washed with 1 CV Buffer W + urea or Buffer C per wash step until the A280 nm was below 0.1 or reached a constant signal. The elution of the target proteins was performed by applying 6 times 0.5 CV of either Buffer BXT + urea to the Strep-Tactin®XT column or Buffer E + urea to the Strep-Tactin® column. His_6-tagged-CP was eluted from the Ni-NTA column using Buffer D1 (100 mM NaH_2PO_4, pH 5.9, 10 mM Tris/HCl and 8 M urea) and in a second elution step with Buffer D2 (Buffer D1 at pH 4.5).

All elution fractions were analyzed by SDS-PAGE and the protein concentration was determined using a Nanodrop photometer.

Results

Highest protein purity despite physiological buffer conditions

In a first series of experiments the fluorophore mCherry and the metabolic GAPDH were purified either as His_6-tag or Twin-Strep-tag® fusion protein under native conditions. Both purifications were performed under the tag-specific buffer conditions, including 300 mM NaCl and 20 mM imidazole for the His_6-tag purification, and 150 mM NaCl for the Twin-Strep-tag® purification, whereas the latter salt concentration can be claimed as physiological. SDS-PAGE analysis revealed that purification of mCherry and

GAPDH with the Strep-Tactin®XT:Twin-Strep-tag® system led to a higher purity of the target proteins than the Ni-NTA:His_6-tag system (Fig 2.). Lower bands in the Twin-Strep-tagged mCherry elutions were identified as degradation products according to Western blot analysis. Therefore, they are no unspecific impurities.

Strep-Tactin®XT and Twin-Strep-tag® allow efficient purification under denaturing conditions

In a second series of experiments the customer protein was purified under denaturing conditions thereby comparing the performance of the Strep-Tactin®XT:Twin-Strep-tag® system to the Ni-NTA:His_6-tag system. In addition, the Twin-Strep-tag® was also used in combination with common Strep-Tactin®.

In the presence of 8 M urea no protein eluted from the Ni-NTA column if Buffer D1 was used (Fig 3.). Hence, a second elution step was required using a pH shift to pH 4.5 to elute the target protein (Buffer D2). When using Strep-Tactin® purification was barely possible in the presence of 4 M urea since only marginal amounts of the customer protein were eluted. In contrast, Strep-Tactin®XT allowed the purification of the target protein even at higher concentrations of urea (6 M) thereby also ensuring a higher purity than the Ni-NTA:His_6-tag system.

Conclusions

The newly engineered Strep-Tactin®XT has an improved binding affinity to Twin-Strep-tag®. This enables high protein purities under both native and denaturing conditions. When using native conditions IBA's 3rd generation Strep-tag® system ensures protein purities which are superior to the His-tag system. Simultaneously, Strep-Tactin®XT preserves all benefits of common Strep-Tactin®, e.g. physiological buffer conditions, mild elution and regeneration of the resin for reuse. Besides this outstanding performance the Strep-Tactin®XT:Twin-Strep-tag® system now offers the possibility to efficiently purify proteins under denaturing conditions. In contrast to Strep-Tactin®, which is not suited for the latter purpose, Strep-Tactin®XT was successfully used for the purification under denaturing conditions. For concentrations up to 6 M urea, Strep-Tactin®XT ensured purer protein than the Ni-NTA:His_6-tag system.

The Strep-Tactin®XT:Twin-Strep-tag® system allows simple one-step elution of the target protein. In contrast, the His-tag system requires the usage of two different buffers since a pH shift is required to promote efficient elution. The absence of imidazole in Strep-Tactin®XT buffers has further advantages. First, there is no interference during absorption measurements in the 260/280 nm range and secondly, desalting or dialysis steps for removal of the imidazole are unnecessary.

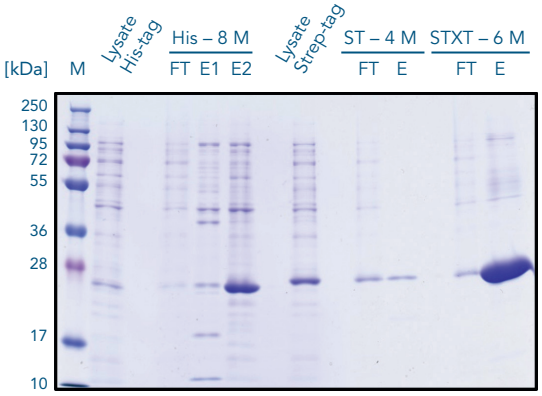

Fig 3. SDS-PAGE analysis of the purification under denaturing conditions of a customer protein comparing the Ni-NTA:His_6-tag system and Strep-Tactin®XT:Twin-Strep-tag® system. M=Marker; His=His_6-tag; ST=Strep-Tactin®; FT=flow through, E=Elution. Given concentrations indicate the used urea concentration. For experimental procedure see text.

References

1. Palmer I, Wingfield PT, 2004: *Curr Protoc Protein Sci.* 38:6.3:6.3.1–6.3.18. Preparation and Extraction of Insoluble (Inclusion-Body) Proteins from *Escherichia coli*.

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Version AN02-17-0002