Application Note

STREP-TACTIN®XT HIGH CAPACITY

High capacity meets high affinity

Introduction

Strep-Tactin®XT is the high affinity resin for the purification of Strep-tag® fusion proteins, providing binding affinities in the picomolar range for Twin-Strep-tag® (TST) while still maintaining binding reversibility and mild recovery of immobilized proteins. In order to maximize the protein binding capacity of Strep-Tactin®XT, a high capacity version of this matrix has been developed, which allows the purification of larger amounts of pure protein requiring small resin bed volumes. This leads to highly concentrated elution fractions and a cost efficient purification procedure. In particular, Strep-Tactin®XT high capacity provides superior performance for the purification of Strep-tag® fusion proteins from diluted cell extracts and allows intensive wash procedures with large volumes of wash buffer, which can result in low protein recovery when using traditional Strep-Tactin® high capacity. Thus, the XT version of Strep-Tactin® high capacity eliminates any known drawbacks of the Strep-Tactin® matrix by combining the enhanced affinity performance of Strep-Tactin®XT with the excellent binding capacity of conventional Strep-Tactin® high capacity. Strep-Tactin®XT high capacity is the new standard for efficient one step affinity purification of Strep-tag® fusion proteins.

This Application Note compares Strep-Tactin®XT high capacity with Strep-Tactin® high capacity with respect to their binding capacities and the costs per milligram purified protein. By purifying proteins with different properties, we demonstrated that Strep-Tactin®XT high capacity is the most efficient resin of the Strep-Tactin® family.

Results and discussion

Strep-Tactin®XT high capacity was developed to combine the extraordinary affinity of IBA's Strep-Tactin®XT 4Flow® with the inherently large protein binding capacity of Strep-Tactin® high capacity. The maximum binding capacity of Strep-Tactin®XT high capacity (~31 mg/ml) is around 1.6x higher compared

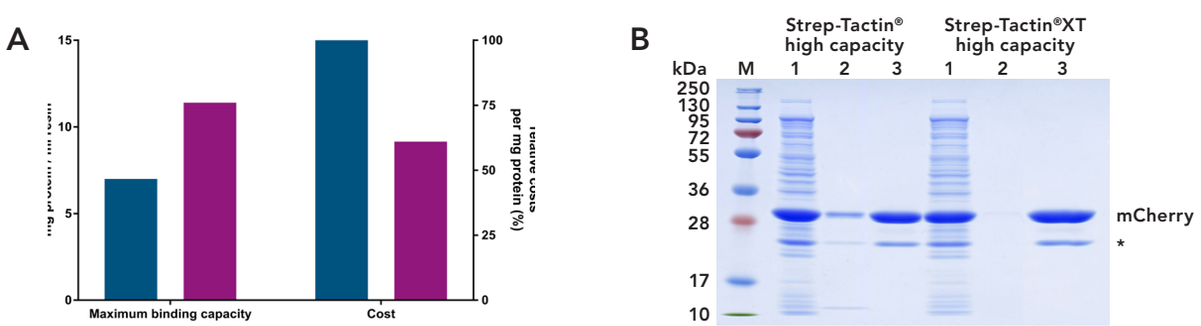

Figure 1. Key advantages of Strep-Tactin®XT high capacity

(**A**) Maximum binding capacities and related costs of Strep-Tactin®XT high capacity and Strep-Tactin® high capacity. Prices were calculated based on the binding capacities of all columns and bulk formats and their corresponding list prices.

(**B**) SDS-PAGE analysis of mCherry-TST purified on Strep-Tactin® high capacity or Strep-Tactin®XT high capacity. 1: lysate; 2: last wash; 3: elution. * natural degradation product generated during maturation of mCherry.

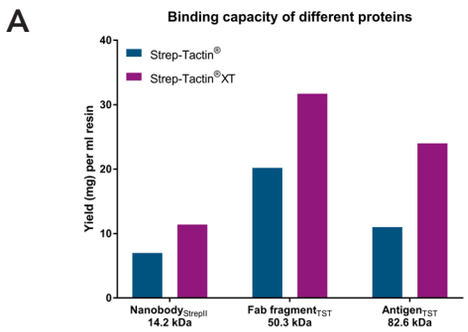

Figure 2. Maximum binding capacities of different proteins The maximum binding capacities of Strep-Tactin® or Strep-Tactin®XT high capacity was determined with gravity flow columns for three differently sized proteins fused to either Strep-tag®II or Twin-Strep-tag®.

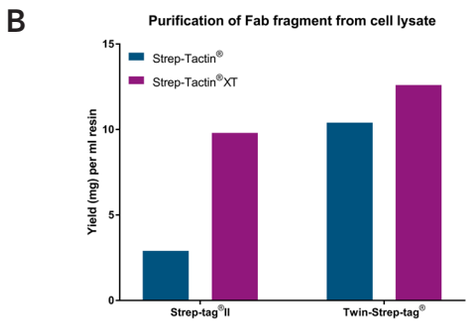

Figure 3. Purification of CD31 Fab fragment from diluted cell lysate A Fab fragment fused to either Strep-tag®II or Twin-Strep-tag® was added to cell lysates at a concentration of 0.6 mg/ml and subsequently purified using Strep-Tactin® or Strep-Tactin®XT high capacity FPLC columns.

to conventional Strep-Tactin® high capacity (Fig. 1A). This significant increase in the binding capacity makes the new Strep-Tactin®XT high capacity the most cost efficient resin among all Strep-Tactin® resins: the price is up to 36% lower per milligram purified protein than for Strep-Tactin® high capacity. In addition, Strep-Tactin®XT high capacity inherits the well-known Strep-tag® purity of up to 99%. Furthermore, it prevents the unwanted leakage of the target protein during the wash steps (Fig. 1B).

As proof-of-principle, maximum binding capacities of three different proteins containing either Twin-Strep-tag® or Strep-tag®II were determined for both Strep-Tactin® or Strep-Tactin®XT high capacity (Figure 2). Strep-Tactin®XT high capacity showed the highest binding capacity for all tested proteins (1.6-2 fold higher compared to Strep-Tactin®). In another experiment, a Fab fragment fused to either Twin-Strep-tag® or Strep-tag®II was added to cell lysates at a concentration of 0.6 mg/ml. The amount of purified protein was more than 3-fold lower for the Strep-tag®II-fused Fab fragment if Strep-Tactin® was used (Figure 3). This could partially be rescued with a Twin-Strep-tag®, which has a higher affinity to both resins. Nevertheless, using Strep-Tactin®XT high capacity resulted in the highest yield independent from affinity tag.

In summary, the results demonstrate that compared to other Strep-Tactin® resins, Strep-Tactin®XT high capacity provides superior performance and cost efficiency as well as highest protein binding capacities for Twin-Strep-tag® and Strep-tag®II proteins independent of the raw material. Thus, it is the perfect system for one-step affinity purification.

Material and methods

The maximum binding capacity of Strep-Tactin® 4Flow® high capacity and Strep-Tactin®XT 4Flow® high capacity were determined with 1 ml gravity flow columns. For sample preparation, purified proteins were added to Buffer W. The concentrations were adapted to 3 mg/ml for the tested nanobody and Fab fragment and to 5 mg/ml for the tested antigen. The protein solution was applied to the columns in 1 ml steps until no additional protein was bound to the column anymore, i.e. the amount added to the column was equal to the amount in the flow through. Afterwards, columns were washed with Buffer W and proteins eluted with Buffer E (Strep-Tactin®) or Buffer BXT (Strep-Tactin®XT). The concentration of all proteins was determined by NanoDrop measurements.

To test the purification efficiency of a low concentrated protein from cell lysate, purified Fab fragment was added to *E. coli* lysate at a concentration of 0.6 mg/ml. Proteins were purified from 22.5 ml sample using 1 ml Strep-Tactin® or Strep-Tactin®XT high capacity FPLC columns on an Äkta Start system (flow rate: 1 ml/min). Unbound proteins were washed away with Buffer W (15 – 30 ml). Proteins were eluted with 15 ml Buffer E (Strep-Tactin®) or 15 ml Buffer BXT (Strep-Tactin®XT). Protein concentrations were determined with NanoDrop or via Äkta-integration.

Abbreviations

StrepII: Strep-tag®II; TST: Twin-Strep-tag®;

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