iba Solutions For Life Sciences

Protein Production & Assays

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 recently, 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, the new 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 new 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 (STXT HC) with Strep-Tactin® high capacity (ST HC) with respect to their binding capacities and the costs per milligram purified protein. We focused on diluted and concentrated cell extracts demonstrating that Strep-Tactin®XT high capacity is the most efficient resin of the Strep-Tactin® family.

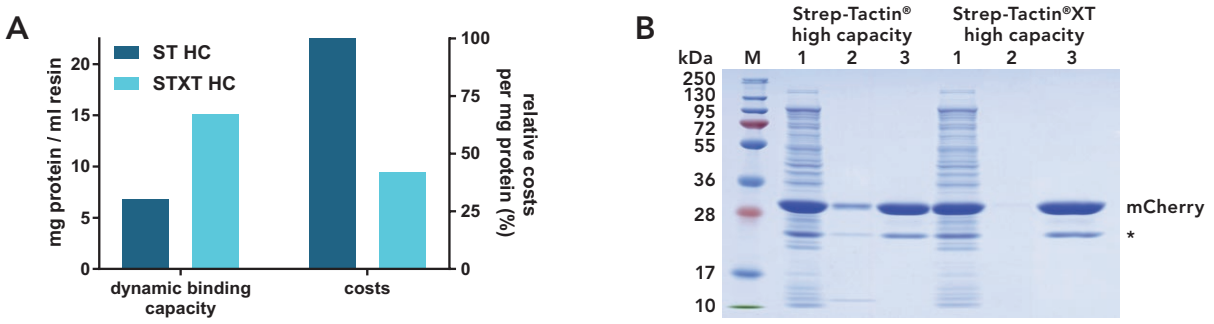

Figure 1. Key advantages of Strep-Tactin®XT High Capacity
(**A**) Dynamic binding capacities and related costs of Strep-Tactin®XT high capacity and Strep-Tactin® high capacity. Prices were calculated based on the binding capacities of all columns and bulk formats and their corresponding list prices.
(**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.

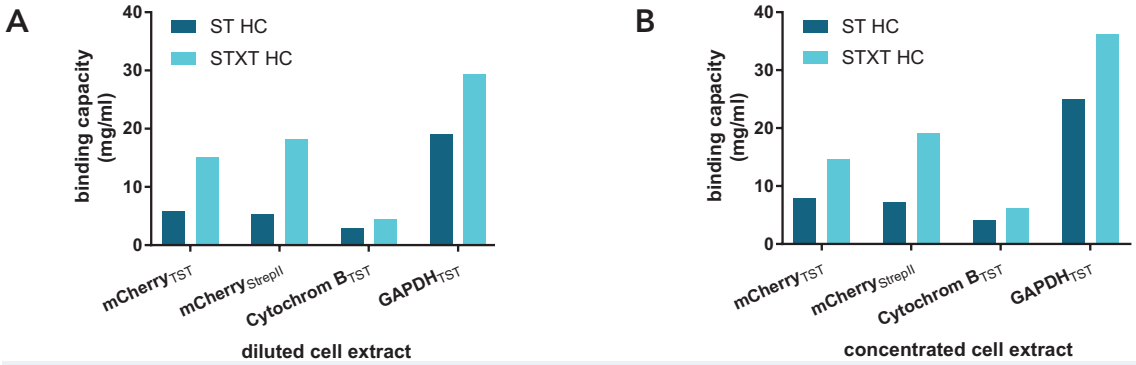

Figure 2. Binding capacities in relation to raw material concentration
(**A**) Diluted (0.4 mg/ml) and (**B**) concentrated cell extracts (3 mg/ml) were purified on Strep-Tactin®XT high capacity and Strep-Tactin® high capacity columns.

RESULTS and DISCUSSION

Strep-Tactin®XT high capacity was developed to combine the extraordinary affinity of IBA's Strep-Tactin®XT with the inherently large protein binding capacity of Strep-Tactin® high capacity. The dynamic binding capacity of Strep-Tactin®XT high capacity (~15 mg/ml) is more than twofold higher compared 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 58% 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, four proteins containing either Twin-Strep-tag® or Strep-tag®II were purified using both resins. Each lysate was applied as diluted cell extract (0.4 mg/ml; Fig. 2A) and as concentrated extract (3 mg/ml; Fig. 2B), respectively. Strep-Tactin®XT high capacity showed the highest binding capacity in all cases. The observed binding capacities were 1.5 – 3.4 fold higher in experiments using diluted cell extracts, and on average 1.8 fold higher when purifying concentrated extracts using Strep-Tactin®XT high capacity. Remarkably, lower protein concentration in the feedstock has no significant influence on the total binding capacity of Strep-Tactin®XT high capacity, independent of the tag used. In contrast, a significantly decreased binding capacity was observed for the diluted cell extracts with Strep-Tactin® high capacity.

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 Dynamic Binding Capacity (DBC) defines the amount of protein, which can be bound on a resin under realistic operating conditions until the leakage of protein in the flow through reaches a value of 10%. The DBC of Strep-Tactin® Superflow® high capacity and Strep-Tactin®XT Superflow® high capacity were determined on an Äkta start. Purified mCherry-TST in Buffer W was adjusted to a concentration of 1 mg/ml. Samples were loaded on Strep-Tactin® high capacity and Strep-Tactin®XT high capacity columns, respectively, until the absorption at A280 nm reached a value of 10% of the raw material. The columns were washed with 10 CV Buffer W followed by elution with 5 CV Buffer E or Buffer BXT. The concentration of all proteins was determined by NanoDrop measurements.

The maximum binding capacities of Strep-Tactin® high capacity and Strep-Tactin®XT high capacity were determined using 0.5 ml gravity flow columns. *E. coli* lysates were spiked with either purified proteins (mCherry-TST and Cytochrome-TST) or lysates (mCherry-Strep®II and GAPDH-TST) to concentrations of 0.4 mg/ml and 3 mg/ml. The prepared samples were loaded on the columns until the flow through contained the respective target protein. Columns were washed eight times with 1 CV Buffer W, followed by elution with 3 CV Buffer E or BXT. Elution fractions were analyzed at 280 nm and by SDS-PAGE.

ABBREVIATIONS

ST: Strep-Tactin®; STXT: Strep-Tactin®XT; HC: high capacity; TST: Twin-Strep-tag®; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; Cytochrome B: Cytochrome b_{562}; CV: column volumes.

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