Purification of *in vivo* biotinylated proteins by using Strep-Tactin®XT

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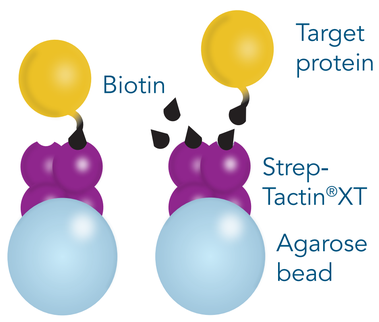
The streptavidin-biotin interaction is a popular choice for applications that require immobilization of proteins but is not suitable for initial protein purification. The high affinity interaction requires harsh elution conditions such as high concentrations of guanidine HCl (GuHCl) or boiling. Consequently, the functionality of a protein is affected, preventing further downstream applications. Therefore, protein purification is usually done via a second affinity tag, most commonly His-tag.

Here, we demonstrate that a second affinity tag is not needed for purifying *in vivo* biotinylated Avi-tag proteins. The key component for this approach is a streptavidin mutant: Strep-Tactin®XT. Its lower affinity to biotin compared to streptavidin allows for mild elution and facilitates the convenient use of biotinylated Avi-tag not only for immobilization, but also for initial purification.

SPECIFICATIONS



Strep-Tactin®XT 4Flow® high capacity

› Max. binding capacity of biotinylated BSA: 13 mg/ml
› Elution with 50 mM biotin
› Regeneration with 100 mM NaOH

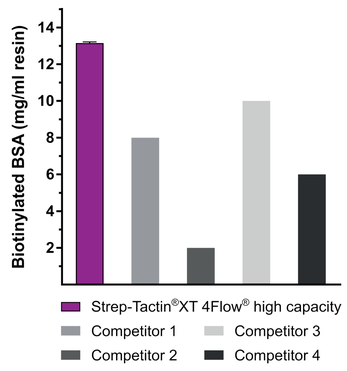
KEY FEATURES

› Mild, non-denaturing elution
› Functional protein
› Exceptionally high purity (> 96%)
› Cost-efficient due to re-usability
› Application of large sample volumes
› Rapid protein purification protocol

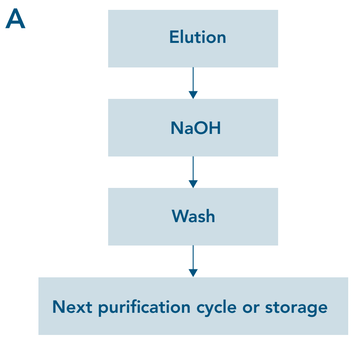
SUMMARY

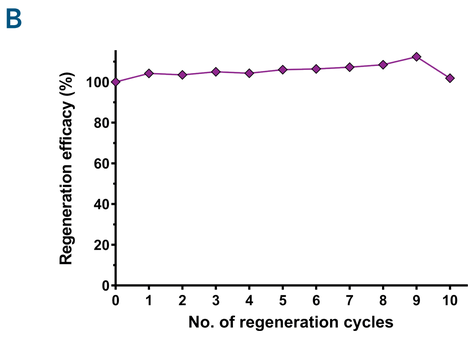
Strep-Tactin®XT 4Flow® high capacity is suitable for purifying *in vivo* biotinylated Avi-tag proteins. Mild elution conditions guarantee functional proteins applicable for subsequent immobilizations and analysis. Due to additional regeneration options, this is a straightforward and cost-effective method for the purification of biotinylated proteins.

HIGH BINDING CAPACITY AS WELL AS QUICK & EASY REGENERATION



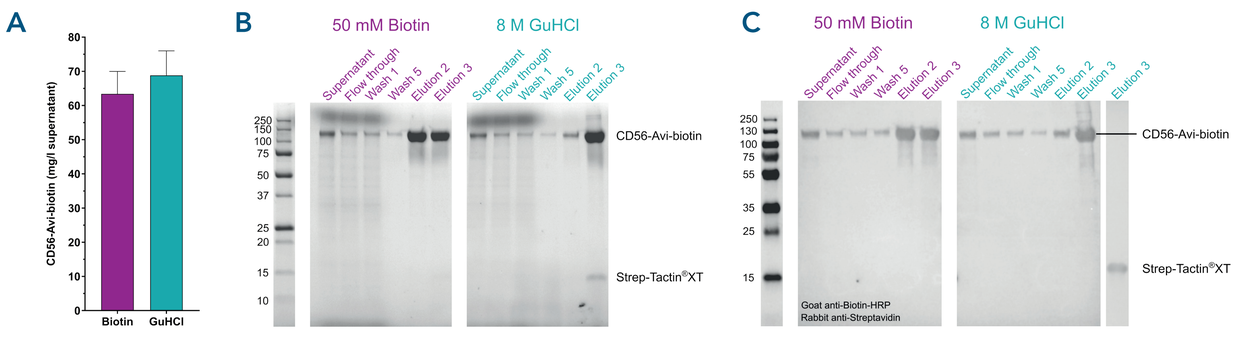
Higher binding capacity than competitors
With 13 mg/ml, Strep-Tactin®XT 4Flow® high capacity has the highest binding capacity compared to competitors, yielding sufficient amounts of protein for further downstream applications.





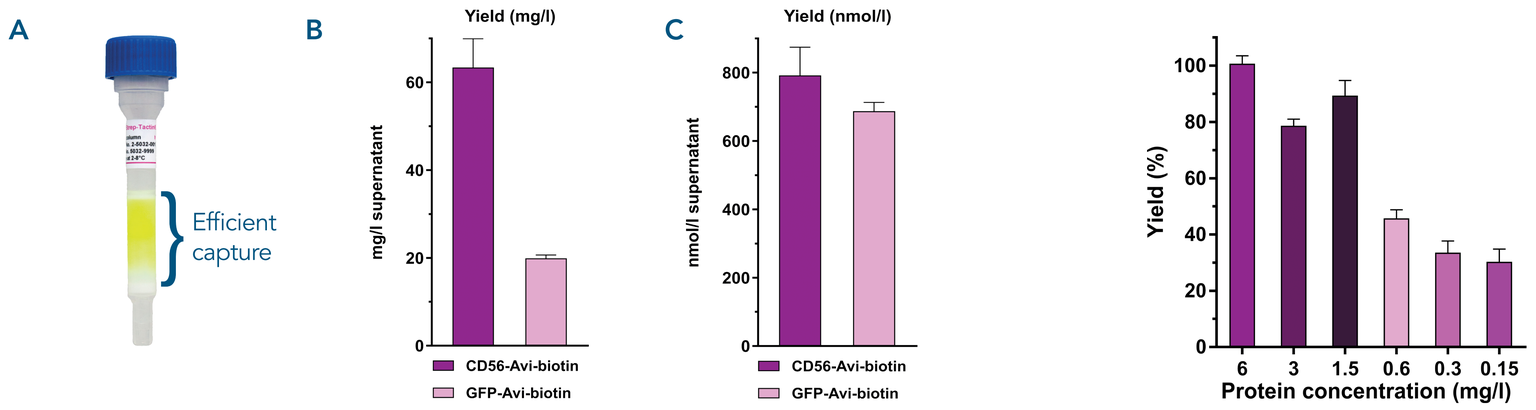
Fast and easy regeneration
Strep-Tactin®XT is easily regenerated with 100 mM NaOH after the final elution step (A). Strep-Tactin®XT 4Flow® high capacity could be reused at least 10 times without any loss in binding capacity for biotinylated BSA (B).

PROTEIN ELUTION IN MILD, NON-DENATURING CONDITIONS



Comparing mild and specific elution with harsh and denaturing elution conditions
In vivo biotinylated CD56-Avi-tag (NCAM1) was purified with Strep-Tactin®XT 4Flow® high capacity resin and either eluted with the specific competitor biotin (50 mM) or under denaturing conditions with GuHCl (8 M). While protein yield was similar for both elution conditions (A), SDS-PAGE showed a contaminating protein after using GuHCl (B), which was identified by Western blot as Strep-Tactin®XT (C). The lower purity of GuHCl elution was confirmed by analytical SEC (D). The denaturing characteristics of the GuHCl elution can affect the structure and functionality, of the target as well as the ligand protein (as shown in D). Therefore a mild and specific elution is advantageous.

HIGH YIELD INDEPENDENT OF PROTEIN SIZE, BUT DEPENDENT ON PROTEIN CONCENTRATION



Efficient purification of differently sized *in vivo* biotinylated proteins
GFP-Avi-tag (29 kDa) or CD56-Avi-tag (82 kDa) were co-expressed with biotin ligase BirA in MEXi cells. *In vivo* biotinylated proteins were directly purified from 100 ml cell culture supernatant with Strep-Tactin®XT 4Flow® high capacity. Strep-Tactin®XT efficiently captured biotinylated GFP (A). Protein purification resulted in 20 mg/l of protein for GFP and 60 mg/l for biotinylated CD56 (B). The overall yield achieved was similar for both, GFP and CD56 (C), indicating efficient purification independent of protein size.

Concentration-dependent protein yield
Different amounts of biotinylated CD56-Avi-tag protein were added to 100 ml cell culture supernatant and purified with Strep-Tactin®XT 4Flow® high capacity. The yield ranged from 100% for the highest concentration tested (6 mg/l) to 30% for the lowest concentration (0.15 mg/l), indicating a concentration-dependent purification efficiency.