# Transient expression in mammalian cells

## A perfect combination of expression and purification

Large quantities of protein are commonly needed in short time for basic research, drug discovery, and toxicological studies as well as for binding and kinetic studies. For transient expression of properly folded and fully active proteins the Expi293™ and the ExpiCHO™ expression systems from Thermo Fisher Scientific are often used. Both systems enable high titer production while simultaneously reducing the expression volume and expression time. To operate effectively in the whole production process it is also crucial to choose an optimal downstream technology for purification. Working with an affinity tag based system is advantageous as a broad range of recombinant proteins can be purified with a single technique without the need for time-consuming optimization.

The most widespread affinity tag system is the His-tag system. However, this system has several drawbacks that can negatively impact purification. Mammalian proteins have a high percentage of His-residues that bind to the metal ions and can cause a high unspecific background. Additionally, using stringent wash conditions with higher imidazole concentrations to improve purity can lead to a loss of the protein of interest due to premature elution. Lastly, high density expression systems using rich media formulations to allow for high protein titers can also impact recovery in the His-tag system, as spent media components can cause nickel to dissociate from purification resins.

## Problem: His-tag purification of mammalian-expressed proteins is challenging

In combination with high density mammalian protein expression, the His-tag system can lead to poor purification results, if conditions are not optimized. In order to achieve pure proteins for downstream applications from the combination of the His-tag system and high density mammalian systems, further adjustments like dialysis of the supernatant, lower media:resin ratios or the use of strip-resistant nickel resins become necessary. Such adjustments require additional optimization time and effort, however, these challenges can be avoided by using the Strep-tag® system. This white paper shows the advantages of the Strep-tag® technology in comparison to the His-tag system as well as the excellent connection of the Strep-tag® technology with two high density mammalian expression systems, the Expi293™ and the ExpiCHO™ expression systems.

In the following experiment, the differences of recombinant protein purification with the Strep-tag® and the His-tag in ExpiCHO™ supernatant are shown. The ExpiCHO™ supernatant was spiked either with mCherry-His-tag (mCherry-His) or with mCherry-Twin-Strep-tag® (mCherry-TST) and the proteins were purified with a standard 1 mL Ni-NTA column or a 1 mL Strep-Tactin®XT high capacity column. During the loading procedure, mCherry-His already leaks from the column whereas mCherry-TST does not, suggesting that there are some components in the spent media that interfere with the His-tag binding. This leads to a loss of mCherry-His and in conclusion to a reduced protein yield. The Twin-Strep-tag®: Strep-Tactin®XT technology in contrast shows a sharp binding in the upper part of the column and no loss of mCherry-TST to the flow through, leading to improved target protein yield.

To determine the yield of recombinant protein that can be achieved with the Strep-tag® technology in combination with the Expi™ systems, purifications with two different test proteins (mCherry and mAb) were performed. It was also tested if the biotin in the Expi™ media influences the binding of the Twin-Strep-tag® to the Strep-Tactin®XT resin.

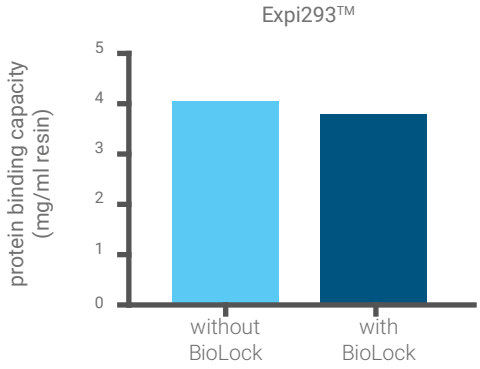

mCherry-His Ni-NTA

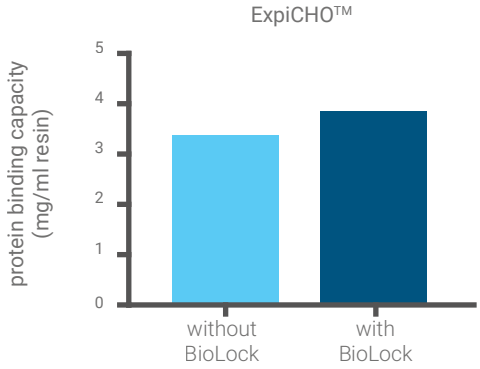
mCherry-TST Strep-Tactin®XT high capacity

## Solution: An alternative purification technology

Scientists who are familiar with the Strep-tag® technology know that biotin is necessary for the elution of Twin-Strep-tagged proteins. The Twin-Strep-tag® is a short peptide that binds specific but reversible to the biotin binding pocket of an engineered streptavidin, called Strep-Tactin®XT. The addition of biotin, which competes for the binding pocket, leads to the elution of the tagged protein. Since the media for mammalian expression systems, often contain biotin, it seems like an unfavorable basis for the Strep-tag® technology at first.

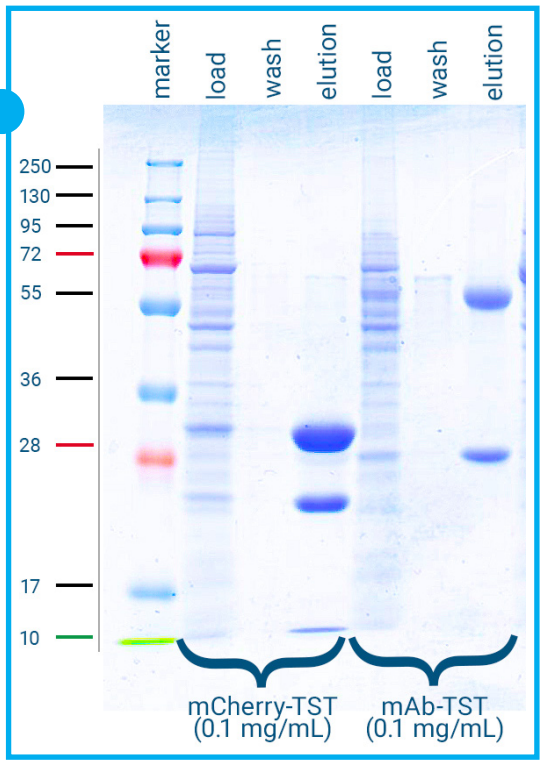
To test if biotin in the Expi™ systems interferes with binding of Twin-Strep-tag®, Expi293™ or ExpiCHO™ supernatants were spiked with mCherry fused to a Twin-Strep-tag® (mCherry-TST). One half of the supernatant with mCherry-TST was directly added to the Strep-Tactin®XT gravity column and the other half was treated with BioLock. BioLock contains avidin, a glycoprotein from egg white, which like streptavidin binds biotin with a high affinity, but does not interfere with the Strep-Tactin®XT resin. Thus the addition of BioLock should bind the free biotin in the Expi™ media. However the experiment shows that there is a comparable yield with and without the addition of BioLock to the supernatant, which leads to the conclusion that the biotin in the media does not interfere with the binding of Twin-Strep-tag® to Strep-Tactin®XT. Consequently the supernatant with Expi293™ and EpiCHO™ can directly be loaded on the Strep-Tactin®XT column without additional preparation steps.



## High recovery and purity - independent of concentration

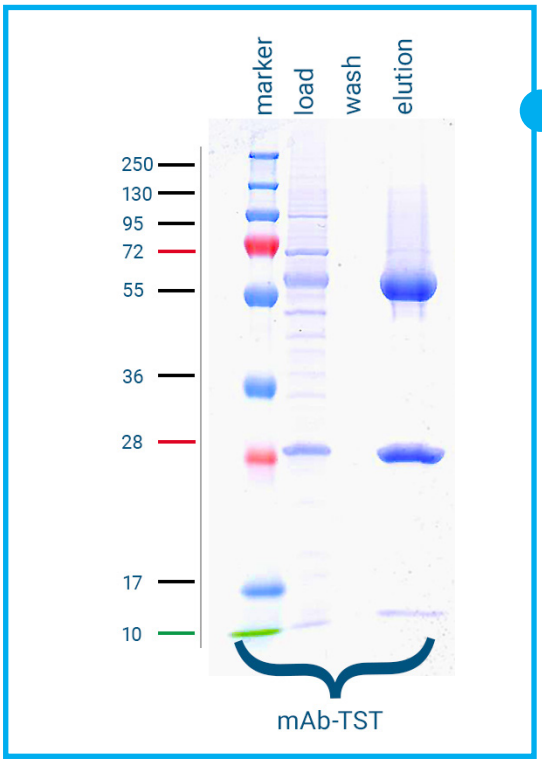
To determine the amount of protein which can be purified from the Expi293™ and ExpiCHO™ media, the supernatants were spiked with test proteins mCherry-TST and mAb-TST to a final concentration of 100 µg/ml and 200 µg/ml. The amount of eluted protein was then compared to the amount of loaded protein (75% of the maximum binding capacity). mCherry-TST and mAb-TST could be recovered in the Expi293™ to 90-100 %. With ExpiCHO™, the amount of recovered protein was 10 % lower. The purity of the eluate was shown on a SDS-PAGE gel. Both proteins could be purified up to 98 % purity. This experiment shows, that proteins can be purified in a high purity and with unparalleled recovery from Expi™ media with the Strep-tag® technology.




## Experiment under real conditions

To also test the performance under real conditions, Expi293™ cells were transfected with a vector coding for a rabbit mAb-TST that is secreted to the cell culture supernatant.

The cells were cultivated until they reached a viability of 75 %. The supernatant with the secreted protein was then harvested and the whole amount (30 ml) was loaded on a 1 ml Strep-Tactin®XT Superflow high capacity gravity column. With the Strep-tag® technology 2,3 mg of mAb could be purified with a high purity. This experiment under real expression conditions demonstrates that proteins can be purified with the Strep-tag® technology in combination with the Expi™ system.



# An ideal combination

In contrast to the His-tag system, proteins can be purified with an outstanding recovery and purity from high density mammalian supernatants due to the high specificity of the Strep-tag® technology. For most downstream applications, the removal of the Twin-Strep-Tag® after purification is unnecessary, because of its small size. As the results demonstrate, in comparison to the His-tag no additional optimization like desalting of the supernatants or pre-purification before the main purification process is required. This concludes, that high density mammalian supernatants, such as those of the Expi expression system, can be added directly to the Strep-Tactin®XT column without any manipulation.

The same purification protocol can be used for different expression hosts and the Strep-tag® technology is also compatible with many reagents like high salts, metal ions, chelators and reducing agents. The Strep-tag® technology is suitable for different protein classes like membrane proteins, antibodies and metal ion containing proteins, leading to improved purity compared to His-tag purification. Finally, the data shows how straightforward and conveniently recombinant proteins can be purified from high density mammalian expression systems, when combined with an appropriate technology like the Strep-tag® technology.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