Protein Production & Assays

Application Note

PROTEIN PURIFICATION FROM EXPI SUPERNATANTS

How the use of the Strep-tag® technology facilitates purification

Introduction

During the last years Thermo's Expi expression systems (Expi293™, ExpiCHO™ and ExpiSf9™) have become the gold standard for transient production of recombinant proteins in mammalian and insect cells. All Expi systems provide cell culture media that supports high cell density cultivations and, thus, high yields of recombinant proteins. To achieve this, the media supplements were adjusted as much as possible to the expression host. However, some of these supplements interfere with the purification of His-tag fusion proteins because they lead to a leakage of nickel ions from His-tag purification resins resulting in lower recovery of the target protein. An alternative to the His-tag purification system is the Strep-tag® technology. The Strep-tag® technology was proven to be highly compatible with the Expi293™ and ExpiCHO™ system. In the following case study, we demonstrate the effect of the aforementioned Ni-leakage by comparing the recovery upon purification of His-tag and Twin-Strep-tag® fusion proteins from Expi293™, ExpiCHO™ and ExpiSf9™ supernatants. Large sample volumes were applied to a conventional Ni-NTA resin (Ni-NTA Sepharose™ FastFlow 6), a Ni-NTA resin that is claimed to be resistant against the described Ni-leakage effect (Ni-NTA Sepharose™ Excel) and the newest version of the Strep-Tactin®XT resin known as Strep-Tactin®XT 4Flow® high capacity.

Materials

- Strep-Tactin®XT 4Flow® high-capacity resin
- 1x Buffer W, wash buffer for Strep-Tactin®XT (100 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA, pH 8)
- 10x Buffer BXT, elution buffer for Strep-Tactin®XT (100 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA, 50 mM, pH 8)

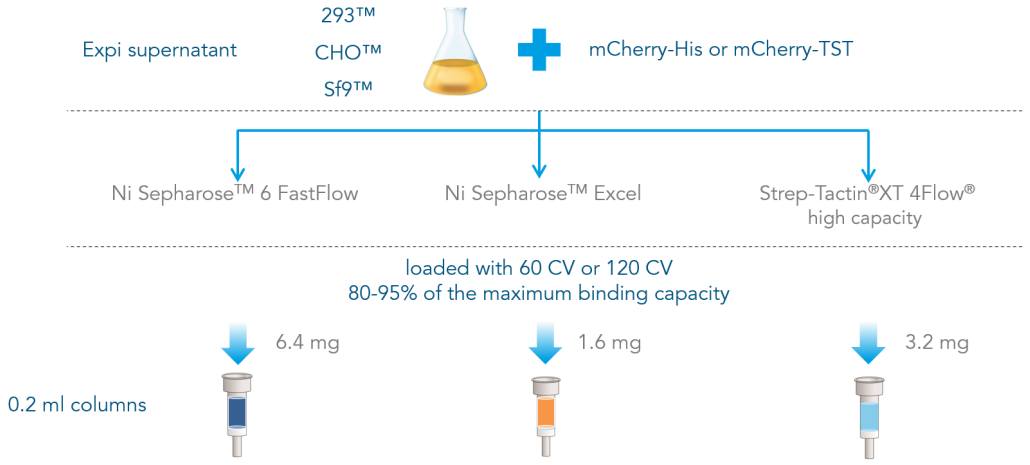

Fig 1. Experimental set up for protein purification from spiked Expi media. ExpiCHO™, Expi293™, and ExpiSf9™ (12 or 24 ml) were spiked with mCherry-TST or mCherry-His. Spiked protein amounts equate to 80-95% of the resins maximum binding capacity. Protein purification occurred with 0.2 ml gravity flow columns packed with Ni-NTA Sepharose™ FastFlow 6, Ni-NTA Sepharose™ Excel or Strep-Tactin®XT 4Flow® high capacity.

- Ni-NTA Sepharose™ FastFlow 6
- Ni-NTA Sepharose™ Excel
- Ni-NTA binding buffer (20 mM NaH_2PO_4, 0.5 M NaCl, pH 7.4)
- Ni-NTA elution buffer (20 mM NaH_2PO_4, 0.5 M NaCl, 500 mM imidazole, pH 7.4)

Methods

Preparation of supernatants

Cell culture supernatants of Expi293™, ExpiCHO™ and ExpiSf9™ cells were harvested, clarified and sterile filtrated. Purified mCherry, fused either to a C-terminal Twin-Strep-tag® (mCherry-TST) or a 6xHis-tag (mCherry-His) were spiked into 12 ml and 24 ml of Expi supernatants. The protein amounts that were spiked in each sample correspond to a total protein load of 80-95% of the resin´s maximum binding capacity. A summary of the experimental set up including the protein loads for each resin is shown in **Figure 1**.

Strep-Tactin®XT 4Flow® high capacity column purification

Strep-Tactin®XT 4Flow® high capacity column purification was conducted according to the manufacture's manual. Briefly, 0.2 ml Strep-Tactin®XT resin was packed into gravity flow columns and equilibrated with 2 column bed volumes (CV) 1x Buffer W. 12 ml or 24 ml of Expi supernatants were loaded directly on the columns followed by addition of 1x Buffer W until A280 nm signal was low. Protein was eluted with 4 CV 1x Buffer BXT.

His-tag column purification

Ni-NTA column purification was conducted according to the manufacture's manual. Briefly, 0.2 ml Ni-NTA Sepharose™ FastFlow 6 or Ni-NTA Sepharose™ Excel was packed into gravity flow columns and equilibrated with 5 CV Ni-NTA binding buffer. 12 ml or 24 ml of Expi supernatants were loaded directly on the columns. Ni-NTA Sepharose™ FastFlow 6 was washed by addition of 3 x 2 CV 1x Ni-NTA binding buffer and Ni-NTA Sepharose™ Excel was washed by addition of 20 CV 1x Ni-NTA binding buffer. Protein was eluted with Ni-NTA elution buffer.

Protein analysis

Protein concentration was determined using a Nanodrop 2000 spectrometer at 280 nm.

Results and discussion

Impact of sample volume on protein recovery

mCherry-TST and mCherry-His were purified either with Strep-Tactin®XT 4Flow® high capacity or one of the Ni-NTA resins from 12 ml spiked Expi293™, ExpiCHO™ and ExpiSf9™ medium. The highest recoveries from all Expi media were obtained with Strep-Tactin®XT 4Flow® high capacity with 85-89% (**Figure 2**). With Ni-NTA Sepharose™ FastFlow 6 53-71% of the spiked protein were recovered and with Ni-NTA Sepharose™ Excel only 33-40%.

To investigate the potential effect on protein recovery caused by increased nickel leakage after application of higher amounts of medium to the columns, the sample volume was increased from 12 ml to 24 ml. The protein recoveries from Strep-Tactin®XT 4Flow® high capacity resin were not affected by the larger sample volumes and showed the best results regarding the portion of unbound protein and, thus, provided

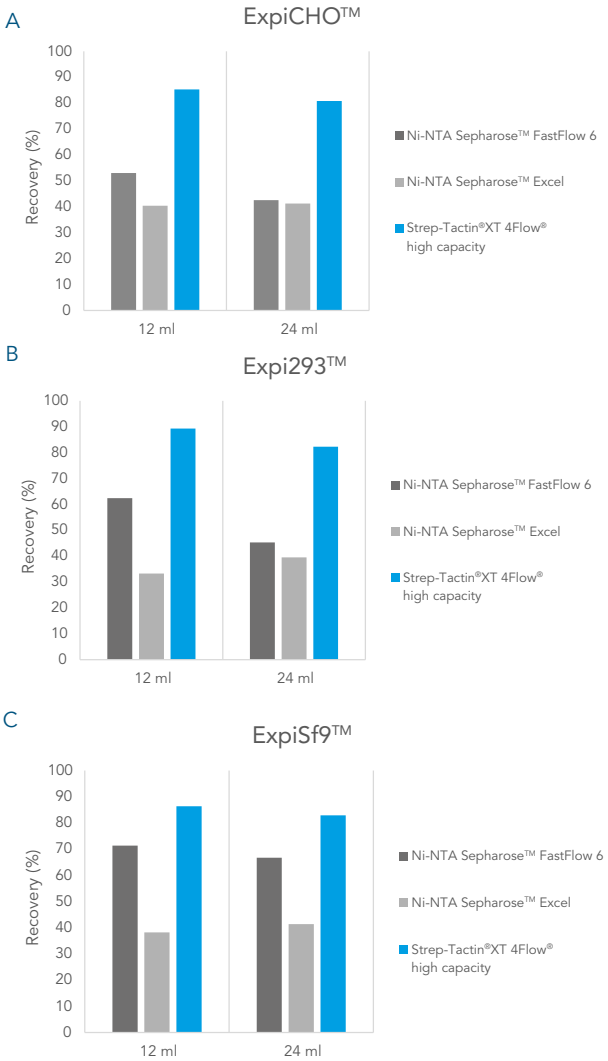

Fig 2. Recovery of mCherry from Expi media. Protein yield after protein purification from 12 or 24 ml spiked ExpiCHO™ **(A)**, Expi293™ **(B)**, and ExpiSf9™ **(C)** medium with Ni-NTA Sepharose™ FastFlow 6, Ni-NTA Sepharose™ Excel or Strep-Tactin® 4Flow® high capacity was determined and and set in relation to the spiked protein amount.

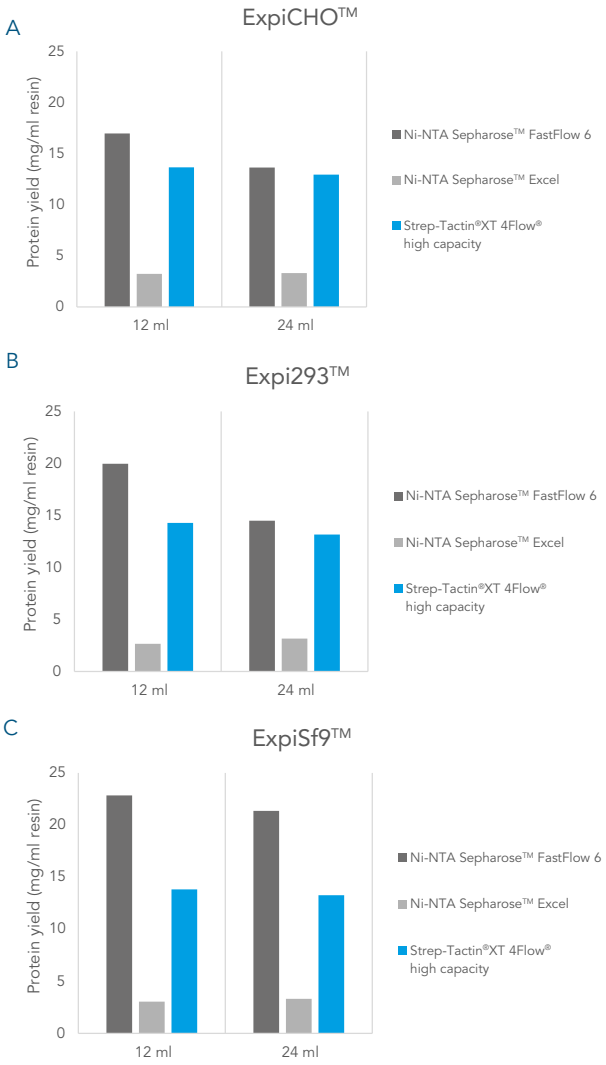

Fig 3. Protein yield of mCherry after protein purification from spiked Expi media. Obtained protein yields of mCherry-His and mCherry-TST after protein purification from 12 or 24 ml spiked ExpiCHO™ **(A)**, Expi293™ **(B)**, and ExpiSf9™ **(C)** were measured and extrapolated for 1 ml resin Ni-NTA Sepharose™ FastFlow 6, Ni-NTA Sepharose™ Excel or Strep-Tactin®XT 4Flow® high capacity.

high protein recoveries in all experiments. The Ni-NTA Sepharose™ Excel was also not affected by the larger sample volume, but recoveries remained low. In contrast, the recovery of mCherry after purification from Ni-NTA Sepharose™ FastFlow 6 was significantly reduced if the sample volume was increased to 24 ml and this effect was most prominent with Expi293™ supernatants (**Figure 2**). Only the supernatants from ExpiSf9™ did not induce a decrease of the recovery from Ni-NTA Sepharose™ FastFlow 6 at larger sample volumes. It can be assumed that the composition of insect cell media differs greatly from the mammalian cultivation media (ExpiCHO™ and Expi293™). Thus, nickel leakage causing supplements might be lower concentrated in the ExpiSf9™ medium.

With the given experimental set up, it can be argued that the low recoveries for Ni-NTA Sepharose™ FastFlow 6 are a result of the higher protein load in these experiments. Although, the protein load was the same based on the percentage of the maximum binding capacity. However, the total amount of bound mCherry was higher or comparable to the Strep-Tactin®XT experiments in Expi293™ and ExpiCHO™ (**Figure 3**). Hence, an important question is if the recovery of Ni-NTA Sepharose™ FastFlow 6 increases if the protein load is reduced to the binding capacity measured in the experiments. Thus, the mCherry purification with 12 ml spiked medium and Ni-NTA Sepharose™ FastFlow 6 was repeated with a protein load of only 50% of the resins maximum binding capacity. A load of 4 mg protein corresponds to the protein yield that was eluted from the Expi293™ experiment. It is worth to mention that the protein concentration in the supernatant is lower due to the reduced protein amount but still higher than in the Strep-Tactin®XT 4Flow® high capacity experiments. Surprisingly, the decreased protein load resulted in even lower recoveries and protein yields compared to the former experiment (**Figure 4**). Consequently, the use of Ni-NTA Sepharose™ FastFlow 6 for the purification of Expi supernatants leads to a substantial loss

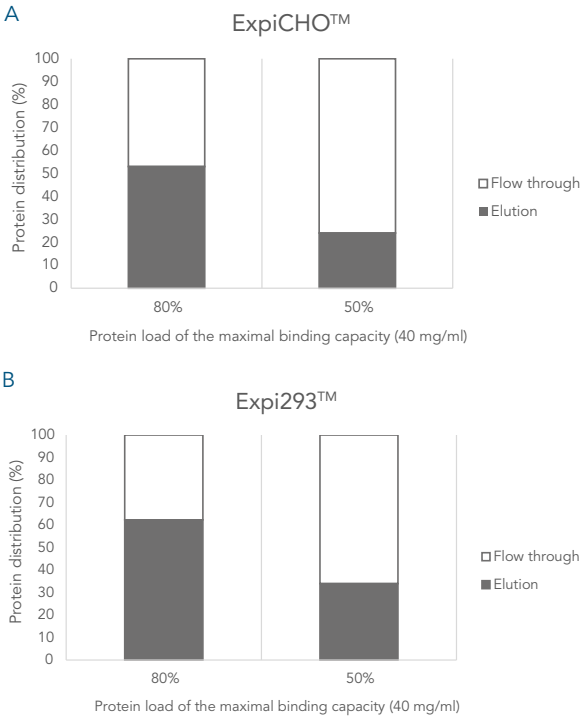

Fig 4. Impact of protein load on Ni-NTA Sepharose™ FastFlow 6. ExpiCHO™ **(A)** and Expi293™ **(B)** were spiked with 80% or 50% of the maximal protein binding capacity of Ni-NTA Sepharose™ FastFlow 6 and, subsequently, mCherry-His was purified. Protein distribution in the flow through and elution fractions was determined.

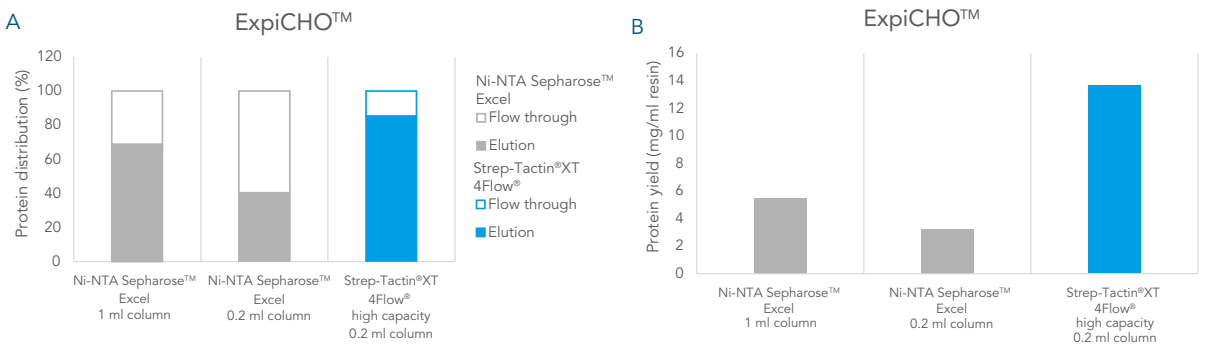

Fig 5. Impact of residence time on protein yield. ExpiCHO™ was spiked with mCherry-TST or mCherry-His. Protein purification occurred with Strep-Tactin®XT 4Flow® high capacity (0.2 ml gravity flow column) or Ni-NTA Sepharose™ Excel (0.2 ml or 1 ml gravity flow column). Protein distribution **(A)** and protein yield **(B)** was determined.

of protein in the flow through fraction independent of the amount of loaded protein. This observation demonstrates that a standard Ni-NTA resin does not provide reliable and predictable purification results.

Residence time influences His-tag purification remarkably

Interestingly, recoveries with Ni-NTA Sepharose™ Excel were only in the range of 36-41% in Expi293™ and ExpiCHO™ although this resin is not prone to the Ni-leakage effect as described in the resin's data sheet and the fact that sample volume did not alter the recovery. Taken in account that the resin volume was only 200 µl and that flow speed cannot be controlled in gravity flow purification, it can be assumed that the residence time was too short for efficient binding. To test this hypothesis, 60 ml ExpiCHO™ were spiked with the same amount of mCherry per ml resin as in the previous experiments and subsequently purified with 1 ml Ni-NTA Sepharose™ Excel to simulate a longer residence time. The recovery increased from 40% to 69% but the recovery at this longer residence time was still lower as that for Strep-Tactin®XT 4Flow® high capacity at the short residence time (**Figure 5**). Total protein yield was also around two-fold lower. These results clearly demonstrate that Ni-NTA resins need longer residence times for efficient binding than Strep-Tactin®XT 4Flow® high capacity and that the Strep-Tactin®XT 4Flow® high capacity is less prone to variations in recovery due to short residence times. It can be assumed that the high binding affinity in the pM range of the Twin-Strep-tag® to Strep-Tactin®XT 4Flow® high capacity is the reason for this robust process.

Conclusion

The results of this case study demonstrate that the combination of Twin-Strep-tag® and Strep-Tactin®XT 4Flow® high capacity outperforms the His-tag/Ni-NTA purification results from Expi293™ and ExpiCHO™ supernatants. Standard Ni-NTA resins exhibit a significant reduction of binding capacity and recovery due to nickel leakage by media supplements. Reduction of the His-tagged protein load did not lead to higher recoveries but further decreased the protein yield. The Ni-leakage resistant His-tag purification matrix avoids the loss of nickel ions during the purification but shows a lower total binding capacity. The protein yields with Ni-NTA Sepharose™ Excel were ~4-fold lower compared to that of Strep-Tactin®XT 4Flow® high capacity. In addition, the lower binding affinity of the His-tag system results in low protein recovery at short residence times also for the Ni-leakage resistant resin. Therefore, the Twin-Strep-tag®/Strep-Tactin®XT 4Flow® high-capacity combination is the preferred system for the purification of recombinant proteins from Expi supernatants.

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