iba

Protein Production & Assays

Application Note

FROM EARLY SCREENING TO LARGE SCALE PRODUCTION

Flexible Scaling of Protein Purification with Magnetic Beads

Introduction

Magnetic bead-based protein purification is a popular choice for small scale and high-throughput applications. It leads to excellent results for samples in the microliter range, even for viscous or debris-containing material. However, the use for larger volumes has been considered inefficient and expensive, forcing a switch to a non-magnetic agarose-based system. This change in method might require optimization steps, resulting in high costs. In addition, the application of larger sample volumes to non-magnetic agarose beads is often a process that takes a long time and requires initial sample clarification steps.

Here, we show that combining our Strep-tag® technology with Sepmag®'s constant and well-defined magnetic force separation eliminates the need for a volume-dependent switch between magnetic and non-magnetic isolation. The Strep-tag® system offers a highly specific tag-ligand interaction with picomolar affinity: Twin-Strep-tag® and Strep-Tactin®XT. Magnetic beads coated with Strep-Tactin®XT (MagStrep® Strep-Tactin®XT beads) have emerged as a powerful tool for screening assays, since they enable robust and efficient purification of proteins with high yield and purity. To demonstrate that Strep-Tactin®XT-coated magnetic beads perform independent of sample size, we tested different volumes **(see Fig. 1)** by linearly upscaling the amount of beads. All conditions resulted in the same high yield and purity, while maintaining short processing times. In combination with the possibility of regeneration, MagStrep® Strep-Tactin®XT beads are a time-saving and cost-efficient solution for protein purification in different developmental stages.

Methods

For the upscaling experiments, 20 µl MagStrep® Strep-Tactin®XT beads were used per ml. After each step, the beads were separated using the sepmag A200ml biomagnetic separator, and the supernatant discarded if not stated otherwise. The scaling was performed from a 2 ml Eppendorf tube (1 ml sample volume) to a 15 ml (7.5 ml sample volume) to a 50 ml Falcon (30 ml sample volume) up to a 250 ml glass bottle

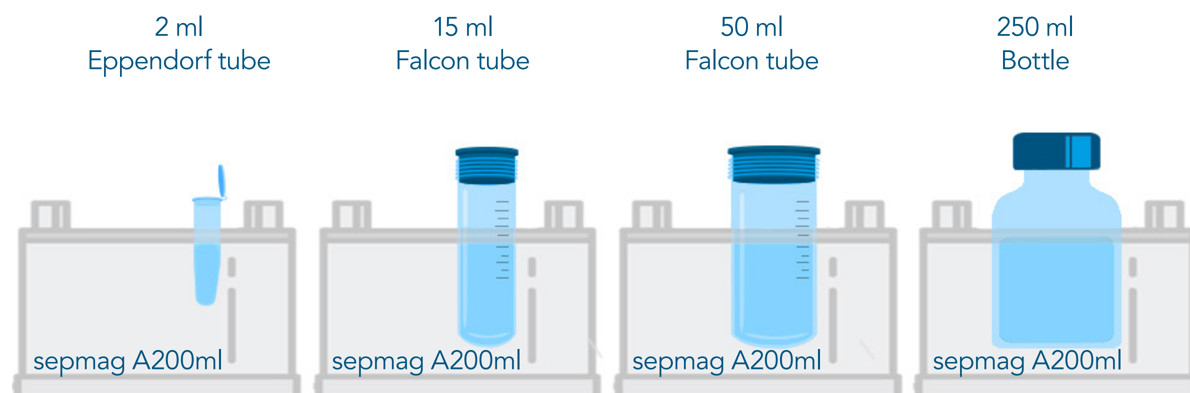

Fig. 1. Experimental overview: A sepmag A200ml biomagnetic separator was used to isolate protein with MagStrep® Strep-Tactin®XT beads from different sample sizes, ranging from 1 ml (2 ml Eppendorf tube) to 125 ml (250 ml bottle).

(125 ml sample volume). MagStrep® Strep-Tactin®XT beads were equilibrated three times using 1.5, 11.25, 37.5 or 187.5 ml Buffer W, depending on the amount of magnetic beads used. The sample containing the target protein was applied to the respective amount of equilibrated beads and incubated for 10 min. Unspecifically bound proteins were removed by washing the beads three times with 1, 7.5, 30 or 125 ml of Buffer W, respectively. Elution of the target protein was achieved by incubating the beads with 1, 7.5, 30 or 125 ml Buffer BXT for 10 min and subsequently collecting the supernatant. Protein purity and concentration were determined via SDS PAGE and absorption at 280 nm, respectively. All experiments were performed at least in duplicates with a regeneration step between each purification. For regeneration, the beads were incubated with 1.5, 11.25, 37.5 or 187.5 ml of a 0.1 M NaOH solution for 2 min and washed twice with 1.5, 11.25, 37.5 or 187.5 ml Buffer W.

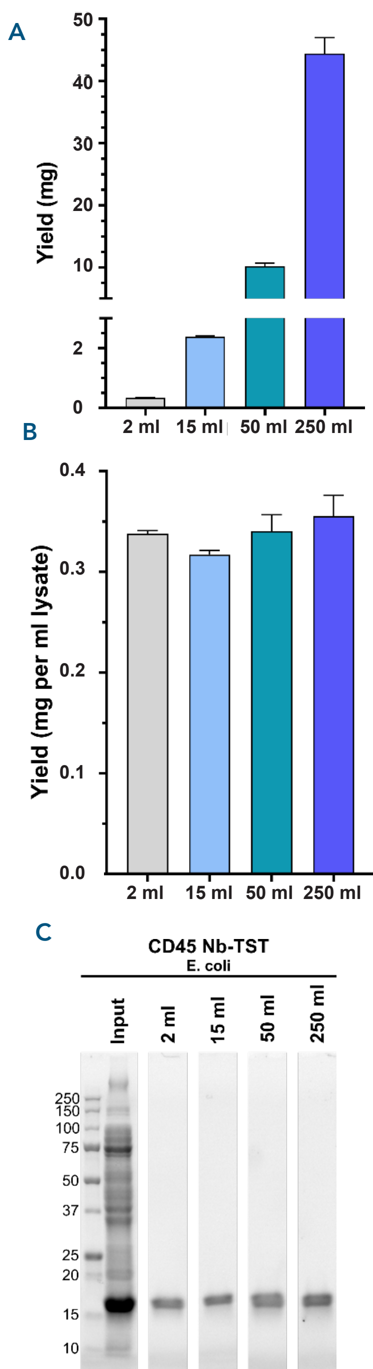

Fig. 2. Scaling up the purification of an αCD45 nanobody fused to a Twin-Strep-tag® from *E. coli* cell lysate using MagStrep® Strep-Tactin®XT beads leads to an increasing amount of isolated protein (A), while the yield per ml lysate remains unaffected (B) and a consistent high purity is achieved (C).

Results & Discussion

Protein yield & purity are independent from sample size

A common concern regarding magnetic beads upscaling is the negative effect on performance and processing time. The development of special biomagnetic separators that generate a consistent magnetic field even through high amounts of material has already optimized the procedure. By using such a magnet (sepmag A200ml), we tested how the performance of our MagStrep® Strep-Tactin®XT beads is affected by increasing sample volumes. We purified an αCD45 nanobody fused to a Twin-Strep-tag® from *E. coli* cell lysates in volumes ranging from 1 ml (in a 2 ml Eppendorf tube) to 125 ml (in a 250 ml glass bottle). The amount of beads was upscaled linearly. Overall, we observed an increasing amount of isolated protein with larger volumes, while the yield per ml lysate remained identical **(Fig. 2 A + B)**. In addition, a purity of at least 95% was achieved in all tested volumes **(Fig. 2C).** Due to the constant magnetic field, even large volumes only needed fewer than two minutes to guarantee separation of the beads after being placed in the magnet. This demonstrates the compatibility of MagStrep® Strep-Tactin®XT beads for various scales, making them an ideal tool for process development, where flexibility in protein purification is essential.

MagStrep® Strep-Tactin®XT beads are compatible with different expression systems

E. coli is a well-established bacterial expression platform, but not always suitable for complex and biologically relevant proteins. Therefore, we investigated if upscaling of magnetic bead isolation not only works for proteins from bacterial lysate **(Fig. 2)**, but also for a mammalian expression system **(Fig. 3)**. To test this, we purified human CD56 antigen (fused to a Twin-Strep-tag®) from different volumes of MEXi-293E cell culture supernatant using MagStrep® Strep-Tactin®XT beads. Similar to *E. coli* lysates, the yielded amount of protein per ml **(Fig. 3A)** and protein purity **(Fig. 3B)**

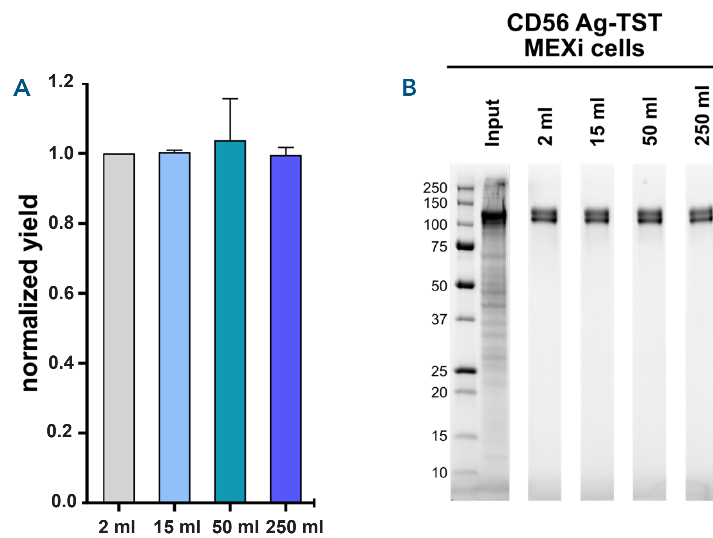

Fig. 3. MagStrep® Strep-Tactin®XT beads offer easy and reliable scalability in different expression systems yielding highly pure protein. Upscaling protein purification from MEXi-293E cell suspension does not change the obtained relative yields (A). Purification of a Twin-Strep-tagged CD56 antigen from MEXi-293E cell suspension resulted in protein purities of at least 95% (B).

were independent of the initial sample volume. These results highlight the robustness and effectiveness of MagStrep® Strep-Tactin®XT beads throughout different expression systems.

MagStrep® Strep-Tactin®XT beads can be reused several times

If proteins are isolated from large sample volumes, magnetic beads must be upscaled accordingly. This translates to high costs, resulting in a decision against magnetic bead isolation. Since the binding of elution agent biotin to Strep-Tactin®XT is reversible, the reuse of MagStrep® Strep-Tactin®XT beads is possible. Regeneration of the beads with 0.1 M NaOH resulted in a stable performance for at least 10 purification cycles **(Fig. 4)**. This way, costs can be reduced even for large scale applications.

Conclusion

Scalability of protein purification is crucial for researchers involved in process development since it allows them to seamlessly transition from laboratory-scale experiments to large-scale production, even for industrial applications. High costs and loss of performance for large volumes are factors that raise concerns for the applicability of magnetic bead-based protein isolation for such a transition. The use of our MagStrep® Strep-Tactin®XT beads in this process eliminates these concerns, since high purity and yield are unaffected by increasing sample volumes. In addition, choosing the right biomagnetic separator (e.g. a sepmag A200ml) is key to guarantee the efficiency and scalability of the purification process. On top of that, bead regeneration greatly reduces associated costs. Conclusively, this system is poised to drive innovation in the field of protein purification, enabling a reliable and adaptable tool that is time-saving and cost-effective.

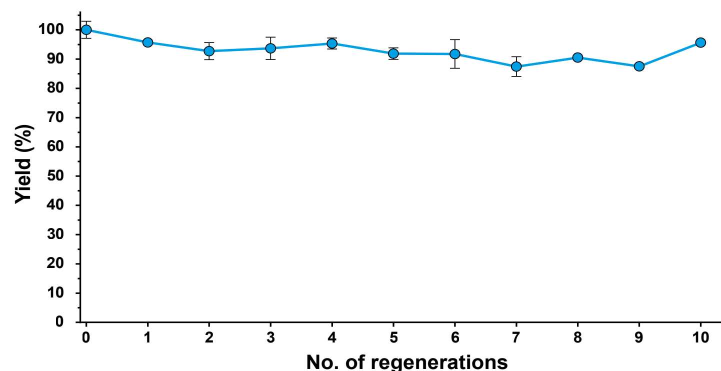

Fig 4. Regeneration of MagStrep® Strep-Tactin®XT beads. MagStrep® Strep-Tactin®XT beads were regenerated using 0.1 M NaOH after each purification. Yield was normalized to mg of protein isolated after the first purification cycle. The binding capacity of the beads remained stable for at least 10 regeneration cycles.

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Version AN02-27-0001