Faster, standardized process development using traceless tag affinity chromatography

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Introduction

Therapeutic proteins must be produced with a very high level of purity, but proteins other than mAbs often don't have an affinity binding partner to facilitate their purification. To be able to standardize and simplify the purification with an affinity step, one strategy has been to add a tag to the recombinant protein. Historically, using a tag has mainly been used for research purposes and not for biotherapeutic purification because tag removal was not traceless, and could generate modifications of the target protein. With Cytiva™ Protein Select™ technology, process development can be standardized with the use of a traceless and self-cleaving tag. This shortens the overall development time while maintaining — or improving — product quality in fewer steps.

Principle

Cytiva Protein Select resin is an affinity chromatography resin for purifying recombinant proteins using the self-cleaving Cytiva Protein Select tag. During an affinity step performed with Cytiva Protein Select resin, the protein self-cleaves from the tag and elutes without residual tag amino acids.

Scan the QR code to watch the animation

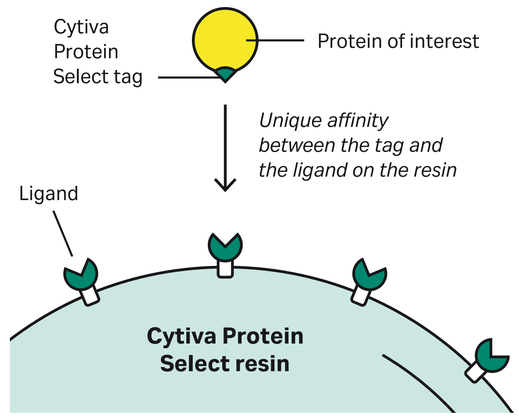


Fig 1. Cytiva Protein Select tag is recognized by the affinity ligand on Cytiva Protein Select resin.

Traceless tag cleavage

Below are the results of mass analysis of a scaffold protein purified with Cytiva Protein Select resin and tag. The mass of the purified protein equals the expected mass after cleavage, confirming that the protein is pure and in native state with no traces of the tag amino acids.

Protein	Mass
Protein with tag	22 141 Da
Expected mass after tag removal	16 876 Da
Mass measured after purification with Cytiva Protein Select resin	16 876.7 Da

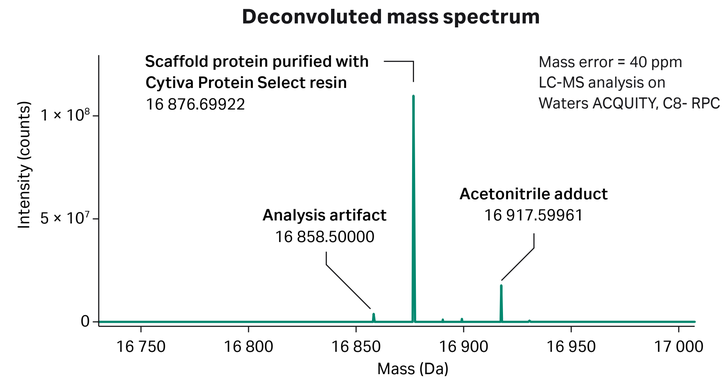


Fig 2. Mass analysis shows no traces of the tag amino acids after cleavage.

Standardized, easier, and convenient process development with higher purity

Part 1: Screening for suitable techniques in small-scale, HiTrap™ formats

We compared the complexity and time requirements of two approaches for purifying a thermostable scaffold protein expressed in *E. coli*.

Requirements: purity ≥ 95%, recovery ≥ 50%

Non-affinity process — 11 weeks development time

Selected protocol

- Step 1: **AIEX resin** — HiTrap Capto™ Q, 5 mL
- Step 2: **Heat treatment** — 65°C, 5 min; Clarification by centrifugation
- Step 3: **HIC resin** — HiTrap Capto Phenyl ImpRes, 1 mL
- **95% purity**

We ran 49 experiments to test various combinations to identify the suitable protocol.

Below are examples of other resins tested:

- **HIC resin** — HiTrap Phenyl Sepharose™ 6FF (Low sub), 1 mL
- **CIEX resin** — HiTrap Capto SP ImpRes,1 mL
- **HIC resin** — HiTrap Phenyl Sepharose 6FF (High sub), 1 mL
- **AIEX MM resin** — HiTrap Capto Adhere, 1 mL

Affinity process with Cytiva Protein Select resin — 3 weeks development time

Selected protocol

- Step 1: **Affinity resin** — HiTrap Protein Select column, 5 mL
- Step 2: **AIEX resin** — HiTrap Capto Q ImpRes, 1 mL
- **98%–100% purity**

We ran 17 experiments to test various combinations to identify the suitable protocol.

Below is an example of another resin tested:

- **HIC resin** — HiTrap Capto Phenyl ImpRes, 1 mL

Fig 3. Development of the small-scale affinity-based process took less time (3 weeks vs 11 weeks for non-affinity process) and was less complex (2 steps vs 3 steps).

AIEX = anion exchange chromatography; CIEX = cation exchange chromatography; HIC = hydrophobic interaction chromatography; MM = mixed mode chromatography

Part 2: Process performance (purity and recovery) at 10 cm bed height

We transferred the best-performing processes from screening (Part 1) to 10 cm bed-height columns and compared their performance.

Non-affinity process AIEX + heat treatment + HIC	Affinity process with Cytiva Protein Select resin
AIEX HiScreen™ Capto Q resin	**Affinity chromatography** Cytiva Protein Select resin in Tricorn™ 10
Heat treatment 65°C, 5 min Clarification by centrifugation	**AIEX** HiScreen Capto Q ImpRes resin
HIC XK 16/10 Capto Phenyl ImpRes resin	
Purity: 91%–92% **Recovery: 57%**	**Purity: 98%** **Recovery: 53%**

Fig 4. Comparison of process performance at 10 cm bed height.

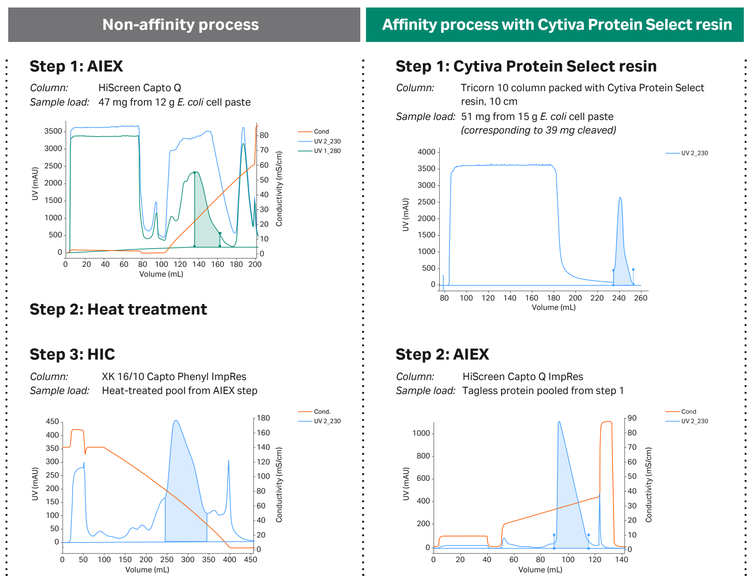


Fig 6. Chromatograms obtained from the different processes.

Facilitating recovery analysis

We used the same protein construct for both processes: we added a 6×His epitope tag upstream of the Cytiva Protein Select tag to facilitate recovery analysis of full-length fusion protein in crude cell extracts.

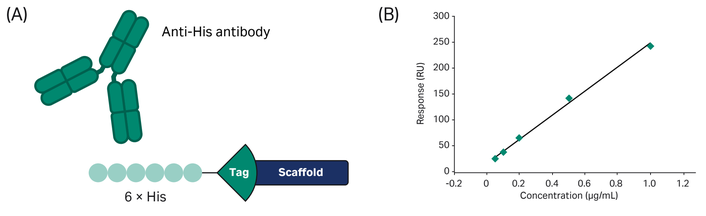


Fig 5. (A) Protein construct with upstream 6×His tag allowing Biacore™ SPR analysis using anti-His antibodies. (B) Calibration curve using purified fusion protein as standard for recovery calculations.

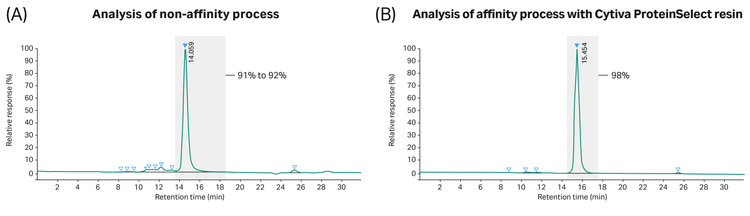


Fig 7. Analytical size exclusion 214 nm on Superdex™ 75 Increase 10/300 GL column. The non-affinity process did not produce the required purity, whereas the affinity-based process did.

Reusability, CIP, and regeneration

After each purification, Cytiva Protein Select resin needs cleaning-in-place (CIP) and regeneration to remove cleaved tag, uncleaved protein, and impurities. We tested an *E. coli* homogenate (Fig 8) and a HEK293 feed (Fig 9) containing a protein tagged with Cytiva Protein Select tag together with different CIP solutions on 1 mL columns. Each purification cycle used a single buffer, a cleavage time of 4 h, and regeneration and CIP for 15 min with the respective CIP solution.

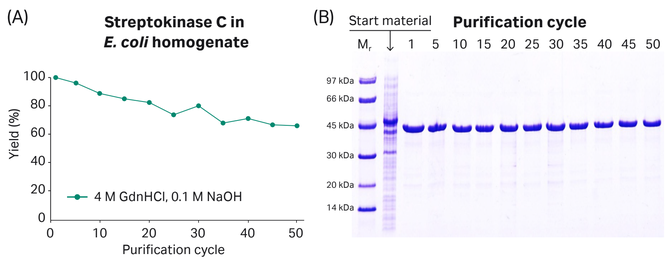


Fig 8. After 50 cycles of *E. coli* homogenate and CIP with 4 M guanidine HCl (GdnHCl), 0.1 M NaOH, the resin retained more than 65% of its initial yield and the purity remained >91% for all cycles. (A) Relative yield in the eluate. (B) Purity according to SDS-PAGE.

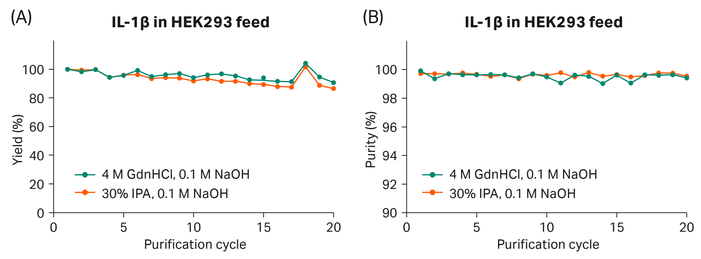


Fig 9. After 20 cycles of HEK293 feed and CIP on two columns with either 4 M GdnHCl 0.1 M NaOH, or 30% isopropanol, 0.1 M NaOH, the resin retained more than 86% of its initial yield and the purity remained >99%. (A) Relative yield in the eluate. (B) Purity according to analytical size-exclusion chromatography (SEC).

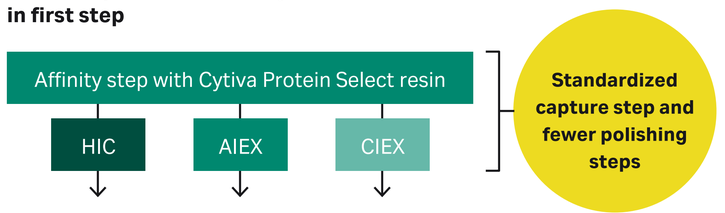
Conclusions

Cytiva Protein Select technology standardizes purification of proteins that do not have an affinity binding partner.

Benefits of this technology include:

- Processes are easier and faster to develop
- Higher protein purity, in fewer steps
- Resin reusability

Platform processes with Cytiva Protein Select resin in first step



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