Elevating Protein Purification Efficiency: Transitioning from Manual Techniques to Automated Platforms

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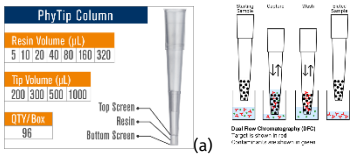
Introduction

Small-scale protein purification is an efficient method for screening samples for protein expression, stability, and functionality as well scaling down process development workflows for drug candidates. This approach conserves samples by minimizing material usage and expediting purification through parallel high-throughput techniques. Common platforms that are well-suited for small-scale protein purification include spin columns, filter plates, magnetic beads, and now PhyTip® Columns. Different workflows have unique purification requirements and challenges. For instance, in screening labs, the primary objective is to isolate substantial quantities of protein for downstream analysis, while in process development, the focus is on optimizing purification methods and refining protocols to ensure reproducibility and facilitate production scalability. As throughput increases, manual platforms often face challenges such as low protein yields, sample handling errors, equipment limitations, and strenuous hands-on time.

Automation can alleviate some of the challenges of manual purification by providing scalability, time efficiency, and compatibility with high-throughput processes. However, scientists seeking to automate their purification process do not want to compromise on quality, robustness, and efficiency. While many automated platforms can alleviate throughput limitations, users can encounter issues such as cross-contamination, capacity limitations, sample-to-sample variation, and reduced flexibility in process development. PhyTip® columns were designed to overcome these barriers to automation. This poster evaluates PhyTip® columns against other common high-throughput purification platforms on these performance criteria.

PhyTip® Columns: Design and Operation

The PhyTip® column technology has been designed to provide high-performance protein purification in a format that allows for complete automation while maintaining a high level of control over the separation process. The high capacity disposable microcolumns are confined within the body of pipette tips by encasing the resin between two inert screens situated at the ends of the tips. The unique design contributes virtually no dead volume to the column, resulting in extremely efficient processing of small sample volumes.



(a) (b)

Figure 1. *PhyTip columns in various tip sizes (a) Schematic of DFC process on PhyTip Column (b).*

PhyTip® columns utilize Dual-Flow Chromatography (DFC) for the purification process, maximizing sample binding to the resin which is optimal for high protein recovery. The DFC mechanism involves a series of sample and buffer aspirations and dispensing sequences directly through the resin-packed tip. DFC effectively captures and releases biomolecules, offering a level of process control that is both automatable and reproducible.

Comparison of Purification Platforms

Capacity Comparison with Ni-IMAC and MabSelect™ Resin

Many factors influence capacity including resin specificity, sample characteristics, and sample-to-resin interactions.

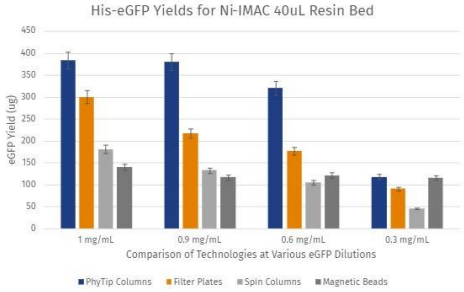


Figure 3. *The eGFP capacity testing on 40 µL of IMAC resin represents the average of triplicates for each sample shown above. The dilutions were prepared with 1x PBS for 4 different starting sample conc.*

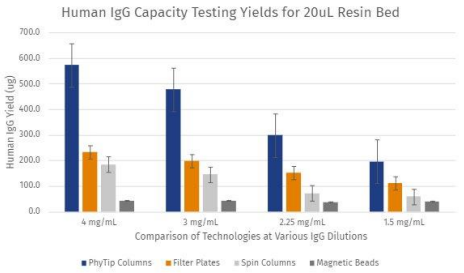


Figure 4. *Human IgG spiked with CHO media for capacity testing on 20 µL of ProPlus (MabSelect Sure™) resin. Each sample was performed in triplicate for each dilution of 4 different starting sample concentrations.*

Biologic samples, such as recombinant proteins, typically exhibit slow binding kinetics making sample residency time on column crucial for efficient binding. PhyTip® columns with DFC allow easy control of this by enabling multiple pass-throughs of the sample through the column. In contrast, spin columns and filter plates have very fast and difficult-to-control sample flow-through speeds, limiting the interaction time between samples and resin. This results in insufficient binding and inefficient protein purification.

Robustness Evaluation

Figure 5 assesses the performance of each protein purification platform in terms of protein yield, result reproducibility and purified protein quality.

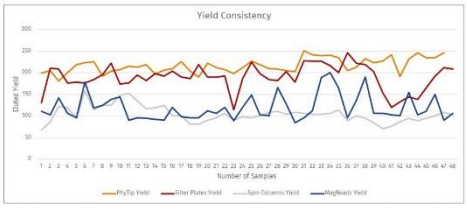


Figure 5. *Consistency yields for Ni-IMAC resin with 40uL volume, tested on 300mg of His-eGFP protein for each respective protein purification platform at standard conditions.*

Among the four platforms that were evaluated, PhyTip® columns delivered the highest yield with the lowest sample-to-sample variation, while also requiring less elution buffer, resulting in higher protein concentrations.

Table 1*. Consistency Experiment Calculations*

Criteria	PhyTip Columns	Filter Plates	Spin Columns	Magnetic Beads
Average Yield (mg)	216.60	189.21	103.86	119.00
STDev.s	16.97	29.42	19.25	29.78
CV (%)	7.83	15.55	18.54	25.02
Percent Recovery	72.20	60.45	34.53	39.56

Elution Efficiency Evaluation

Tip concentrating effect of PhyTip® Columns allows for efficient elution compared to other platforms.

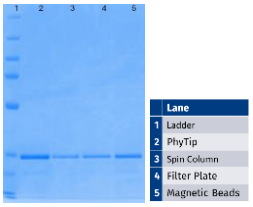


Figure 6. SDS-PAGE gel image.

The unique design of the PhyTip® columns features a thin hydrophilic frit surrounding the packed resin bed, which eliminates virtually all dead volume. A very small amount of liquid aspirated through the hydrophilic frit efficiently covers the entire resin bed, reducing the need for elution buffer. This efficient elution process concentrates the final sample and minimizes contamination compared to other methods.

Process Control

A key benefit of PhyTip® Columns is the ease of method development and fine level of process control. PhyTip Columns can be placed directly on the liquid-handler deck just standard pipette tips. Method development is as easy as adjusting the liquid handler parameters such as flow rate or cycle numbers.

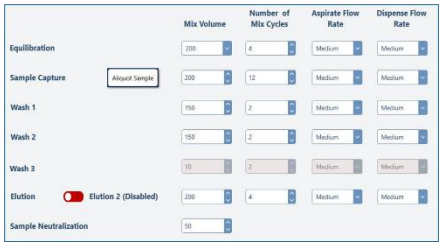


Figure 7. *Screenshot of the parameters set for PhyTip® columns for automated liquid-handling platform. The parameters can be altered to meet specific requirements of the experiment prior to each run.*

Factors to Consider: Automating Protein Purification

Versality: Ability to accommodate various workstations and adapt easily for different proteins and workflows.
Process Control: Ease of method development.
Budget: Cost associated with module integration for automation. This can vary significantly for platforms that are module dependent.
Implementation Time: Time required to integrate and validate the automated workflow for process development.

Table 2*. Comparison of each platform for considering automation*

	Spin Columns	Filter Plates	Magnetic Beads	PhyTip Columns
Versatility	••	•	•	•••
Process Control	••	••	••	•••
Set-up Cost	•	•••	••	•
Implementation Time	•	••	••	•

Summary

- PhyTip® Columns are designed for automation and use Dual-Flow Chromatography (DFC) mechanism for the purification process.
- DFC ensures complete binding of the sample to the resin as well as gentle purification.
- PhyTip® Columns do not require additional modules required or manual column preparation.
- PhyTip® Columns can be placed directly on the Liquid-Handler deck, with process development adjusting parameters such as flow rate or cycle numbers.
- PhyTip® Columns are the ideal choice, providing excellent capacity, consistency, and purity while allowing for easy and reliable process control in method development.