

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

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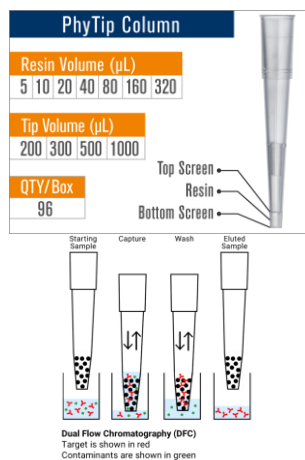
Introduction

Developing effective protocols for sample preparation, extraction, and purification, requires careful consideration of, multiple factors. Optimized protocols should be reproducible, scalable, flexible, gentle on the sample, and efficient. In addition, protocols need to be high-throughput and easily automated to enable efficient processing of multiple samples and different workflows.

To achieve this, several platform options are available, including magnetic beads, filter plates, spin columns, and tip-based technology. This poster evaluates these different platforms for their suitability in high-throughput and automated sample prep, extraction, and purification workflows. We compared performance data across platforms based on yield, binding capacity, reproducibility, and scalability.

Methods

This study compared Biotage® PhyTip® columns (see below), Cytiva filter plates, and ThermoFisher Pierce™ spin columns and magnetic beads for protein purification using Biotage IMAC and ProPlus buffer kits. To evaluate yield consistency, 300 µg of His-eGFP was purified across 48 replicates per platform using 20–25 µL Ni-IMAC resin, measuring recovery and CV values. Binding capacity was assessed in triplicate using His-eGFP (0.3–1.0 mg/mL in BL21) with 40 µL Ni-IMAC resin and human IgG (1.5–4.0 mg/mL in CHO media) with 20 µL ProPlus resin. All samples were eluted in 600 µL. Quantitative analysis was performed via NanoDrop™ (490 nm), with SDS-PAGE utilized for qualitative assessment of purity and binding efficiency.



Comparison of Purification Platforms

Experiment 1: Enhanced Yield and Binding Efficiency

PhyTip® columns achieved significantly higher yields across all protein concentrations tested. For human IgG (1.5–4.0 mg/mL), the MabSelect™ Sure PhyTip® columns maintained efficacy even at the highest concentrations, where competitive platforms showed diminishing returns. Similarly, His-eGFP yields at 1 mg/mL, 0.9 mg/mL, and 0.6 mg/mL were highest using Ni-IMAC PhyTip® columns.

This performance is driven by Dual Flow Chromatography (DFC) - the automated, back-and-forth aspiration and dispensing of the sample over the resin bed. Unlike the "batch binding" seen in magnetic beads or the rapid pass-through of vacuum-driven filter plates, DFC maximizes the residence time and interaction between the protein and the resin, effectively saturating the binding capacity.

Figure 1 (A)

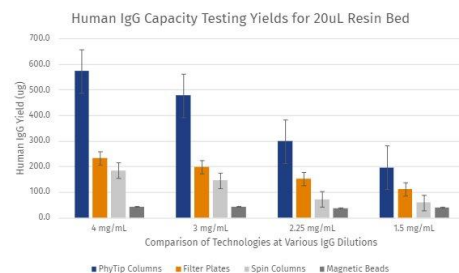


Figure 1 (B)

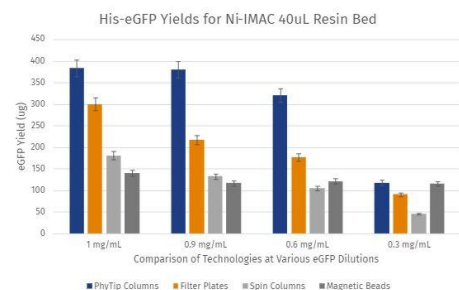


Figure 1. (A) Human IgG spiked with CHO media for capacity testing on 20 µL of ProPlus (MabSelect Sure™) resin. Each sample was performed in triplicate for each dilution of 4 different starting sample concentrations. (B) The eGFP capacity testing on 40 µL of IMAC resin represents the average of triplicates for each sample shown above. The dilutions were prepared with 1x PBS for 4 different starting sample conc.

Experiment 2: Statistical Superiority: Higher Recovery, Lower CV

As shown in Figure 2, PhyTip® columns achieved the highest average yield of 216.6 µg (72.2% recovery) with a remarkably low CV of 7.8%.

- **PhyTip columns: 216.6 µg yield (72.2% recovery); 7.86% CV.**
- **Filter Plates: 189.21 µg yield (60.5% recovery); 15.6% CV.**
- **Magnetic Beads: 119.0 µg yield (39.6% recovery); 25.0% CV.**
- **Spin Columns: 103.9 µg yield (34.5% recovery); 18.5% CV.**

The high reproducibility of PhyTip® technology stems from its independence from external modules like vacuum pressure or centrifugal force, which often introduce well-to-well variability.

Figure 2 (A)

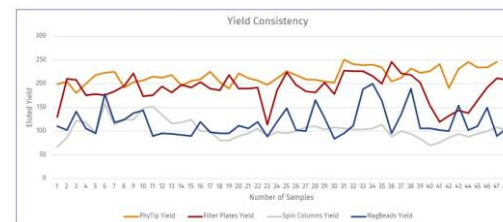


Figure 2 (B)

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

Figure 2. (A) Consistency yields for Ni-IMAC resin with 40uL volume, tested on 300 mg of His-eGFP protein for each respective protein purification platform at standard conditions. (B) Consistency Experiment Calculations

Experiment 3. Efficient Elution and Concentration

SDS-PAGE analysis (Figure 3) confirmed these findings, with Lane 2 (PhyTip®) exhibiting the most intense band. The column's unique design, featuring a thin hydrophilic frit, ensures minimal dead volume. This allowed for highly concentrated elution in as little as 120 µL, whereas other platforms required 200 µL for effective recovery. Consequently, PhyTip® columns not only provide more protein but do so at a significantly higher final concentration.

Figure 3

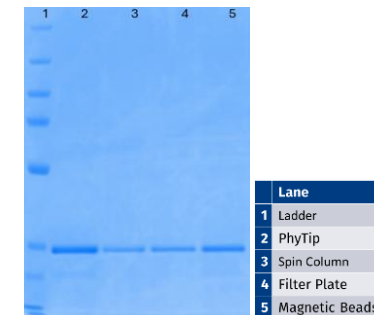


Figure 3. SDS-PAGE gel image highlights final concentration of eluted sample for each respective purification platform. Tip concentrating effect of PhyTip® Columns allows for efficient elution compared to other platforms.

Level of 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.

Factors to Consider: Automating Protein Purification

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

Summary

- » PhyTip Columns are designed for automation and use Dual-Flow Chromatography (DFC) to the perform purification process.
- » DFC ensures complete binding of the sample to the resin as well as gentle purification of the sample.
- » PhyTip Columns do not require additional modules required nor manual column preparation.
- » PhyTip Columns can be placed directly on the Liquid-Handler deck, process development adjusts the Liquid-Handler parameters such as flow rate or cycle numbers.
- » Overall, PhyTip columns are the ideal choice for automating protein purification, outperforming evaluation criteria for capacity, consistency, and purity all while allowing for easy and reliable process control in method development.