



Platforms
and Downstream
Applications

Automating Protein
Purification

Comparing Protein
Purification Yields

Protein Purification
Efficiency and
Yield Consistency

Automating Protein Purification

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Protein Purification Platforms and Downstream Applications

The evolution of protein purification technologies, including automated and high-throughput systems, have enhanced efficiency, scalability, and precision in various downstream applications.

INTRODUCTION

For years, the evolution of protein purification technologies has been driven by a demand to improve the efficiency, resolution, and scalability of protein separation and purification processes. From the early days of column chromatography to the latest advancements in automated and high-throughput systems, each innovation has addressed specific challenges and paved the way for more reliable and productive research and development in both the biotech and biopharma sectors. These advancements have significantly impacted various downstream applications, enhancing the precision and efficiency of processes such as interaction studies, lead screening, cell-based assays, characterization and analytical development, proteomics, and process development.

Advanced purification techniques streamline the production process, making it more efficient and cost-effective. This is important for developing scalable manufacturing protocols.

PROTEIN PURIFICATION THROUGH THE YEARS

1940s: Column chromatography gained popularity in separate complex mixtures of proteins from biological samples.

1960s: Emergence of Liquid-liquid chromatography (LLC) for separating proteins based on solubility.

1970s: Development of high-performance liquid chromatography (HPLC) for higher resolution and faster separations processes.

1980s: Introduction of small-scale chromatography techniques like spin columns, filter plates, and magnetic beads, for rapid purification and parallel processing of multiple small volume samples.¹

1990s: Automated protein purification systems and magnetic bead-based protein purification methods, improved consistency and allowed for high-throughput processing.²

2000s: High-throughput technologies like dual-flow chromatography (DFC), enhanced binding efficiency and allowed for better process control, minimized protein damage, and ensured higher purity and functionality of the resulting purified proteins.³

2010s: Further enhancements in high-throughput and miniaturized purification platforms focus on speed, efficiency, and scalability. These technologies enabled rapid screening and optimization of purification protocols, making them invaluable for the biopharma industry and academic research.



DOWNSTREAM APPLICATIONS FOR PURIFIED PROTEINS

Purified proteins are essential in various downstream applications.

- **Lead Screening:** Efficiently expressing and purifying large libraries of biomolecules is essential for rapid screening processes aimed at identifying potential drug candidates, or functional enzymes or high affinity antibodies.

Technologies that allow for the simultaneous processing of multiple samples are crucial for improving efficiency without sacrificing data quality. Automated purification systems reduce the risk of human error and increase reproducibility. Scalable purification technologies are important for handling varying sample volumes, from small-scale initial screenings to larger scale confirmatory studies. This flexibility allows researchers to adapt their processes as needed, ensuring efficient use of resources.

- **Proteomics:** Purified proteins are essential for identifying potential disease biomarkers. These biomarkers can be used to develop diagnostic tests that

detect diseases at an early stage, improving patient outcomes. Additionally, understanding these biomarkers can lead to the development of targeted therapies that specifically address the underlying cause of diseases.

High purity proteins are essential for analyzing the structure, function and stability of protein-based drugs. This

analysis ensures the drugs are safe and effective for patient use. Advanced purification techniques provide the necessary quality and consistency, which are crucial for regulatory approval and clinical success.

Purified proteins are also used in the production of biopharmaceuticals, where maintaining high quality and consistency is critical. Advanced purification methods help in monitoring and controlling the quality of the final products.

These technologies enabled rapid screening and optimization of purification protocols, making them invaluable for the biopharma industry and academic research.

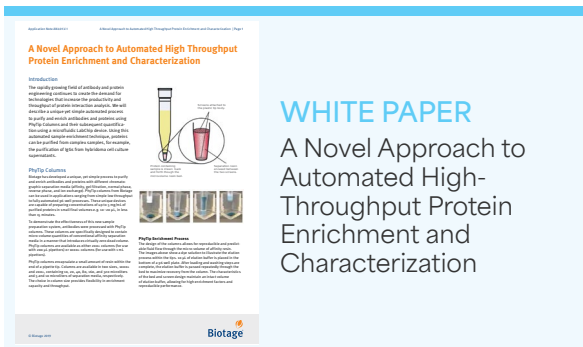
Interaction Studies: Researchers use purified proteins to investigate protein-protein, protein-nucleic acid, or protein-ligand interactions, crucial for understanding the fundamental mechanisms of biological processes and developing therapeutic strategies. Techniques such as surface plasmon resonance (SPR) measure the binding interactions between molecules in real-time without the need for labeling. High purity proteins are crucial for SPR to accurately determine binding kinetics and affinity constants, providing insights into the strength and duration of molecular interactions. Co-immunoprecipitation is used to study protein-protein interactions by capturing protein complexes from cell lysates. The use of purified proteins ensures that the interaction observed is specific and not due to contaminants, allowing for precise identification of interacting partners.

Cell-based Assays: Purified proteins are critical to study their effect on cellular processes using cell cultures. These assays are crucial for understanding how proteins influence various cellular functions and for developing new

therapeutic strategies. Consistent and high-quality proteins are necessary to measure the catalytic activity of enzymes within cells, critical for understanding enzyme function and regulation. Purified proteins are also used in various other cellular studies, such as signaling pathway analysis, receptor-ligand interactions, and cellular response to external stimuli.

High quality proteins ensure that these studies can be conducted with minimal variability and high accuracy.

- **Characterization and Analytical Development:** Purified proteins are necessary for detailed characterization studies, which assess the structure, function and stability of the biomolecule. These studies are essential for understanding the behavior of the protein under different conditions and for optimizing the production process. High-quality proteins are also required for developing various analytical techniques, such as mass spectrometry, used to monitor and control the quality of the production process.



- **Process Development:** Advanced purification techniques streamline the production process, making it more efficient and cost-effective. This is important for developing scalable manufacturing protocols. Screening proteins produced from various expression systems, such as bacteria, yeast, or mammalian cells, for scale with desirable yield and quality, is an important aspect of process development and optimization. Purified proteins play a vital role in developing and optimizing purification protocols, which can then be applied to large-scale production. This development work is essential for creating robust and efficient manufacturing processes.

CONCLUSION

The technology surrounding protein purification has greatly improved over the years to address common analytical challenges, most notably with the introduction of automated protein purification in the 1990s. Even in the modern lab, automation

Especially as the demands of biotechnology and biopharma evolve, leveraging automated systems for protein purification helps ensure downstream applications are continuously streamlined.

is required to significantly enhance throughput, reproducibility, robustness, and scalability, allowing researchers to process more samples efficiently while reducing the risk of human error. It also supports complex purification protocols that may otherwise be time-consuming, labor-intensive, or expensive, making automation an

essential component for scaling processes in both research and industrial settings. Especially as the demands of biotechnology and biopharma evolve, leveraging automated systems for protein purification helps ensure downstream applications are continuously streamlined.

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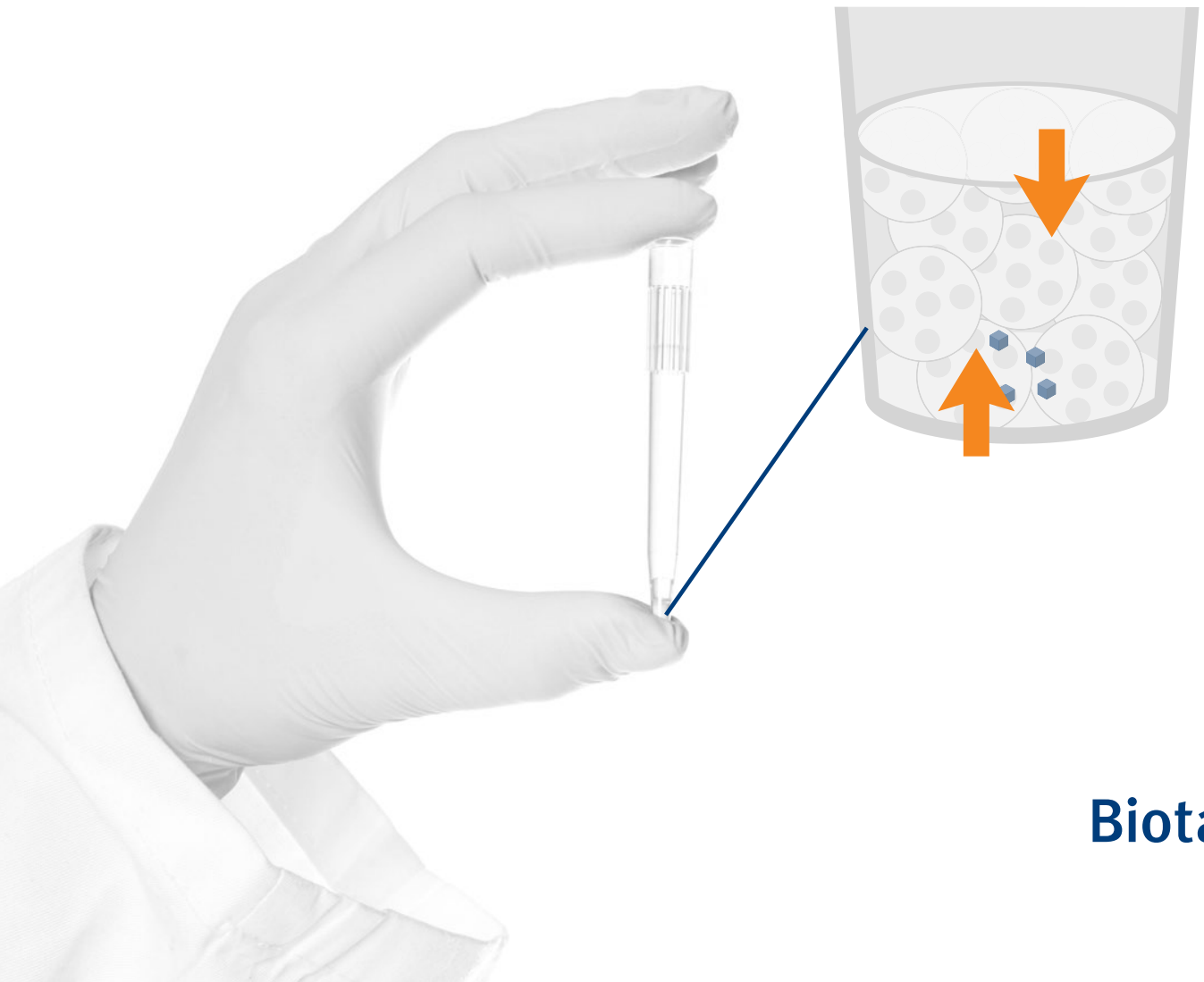
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Optimizing Lab Efficiency: Insights Into Automating Protein Purification Workflows

Benefits, considerations, and challenges of automating protein purification, particularly in high-throughput labs

SME: Gabrielle Kerkow, MBT, Application Specialist, Biotage



What are the benefits of automation?

KERKOW: Automation allows for consistency and reproducibility. It can ensure controlled conditions, leading to reproducible results with improved accuracy and precision. Another benefit is efficiency. Proteins can be purified without user intervention during the run, saving time and reducing manual errors. This is particularly beneficial for sensitive and low-expression proteins. For labs with higher throughput, automation allows for the purification of multiple proteins in parallel, increasing overall throughput. Lastly, automation helps with resource optimization by delegating tedious tasks

to liquid handlers, allowing scientists to focus more on method development and data analysis.

Q When should a lab consider automation when purifying proteins?

KERKOW: Labs should typically consider automation when the benefits outweigh the overhead cost. For high-throughput needs, if your lab is handling a large number of samples, automation can significantly boost throughput and efficiency. Additionally, automation can help achieve consistent results by minimizing human error and variability.

If you have a lab with time constraints, automation systems can process samples faster than manual methods, freeing up time for researchers to focus on other tasks.

If you have a lab with time constraints, automation systems can process samples faster than manual methods, freeing up time for researchers to focus on other tasks. If you are dealing with complex or multi-step purification protocols, automation

can streamline this process and ensure each step is performed accurately. For labs that need to standardize workflows across multiple users or locations, automation guarantees uniformity in procedures and results.

Q What are factors to consider when it comes to automation and liquid handlers?

KERKOW: When considering automation, the first factor to consider is sample throughput. Determine the volume and number of samples you need to process. High-throughput systems can handle multiple samples simultaneously, saving time and increasing productivity.

Low-throughput systems may suffice for smaller labs. The second factor is flexibility. Choose a system that can accommodate various purification methods or workstations. This way, it can easily be adapted for different proteins and workflows. This includes compatibility with different chromatography techniques, such

as affinity, ion exchange (IEX), and size exclusion (SEC). Next consider resource efficiency—evaluate the cost of reagents, maintenance, and the initial investment



in automation systems. Efficient use of resources can overall reduce costs and improve lab productivity.

You also want to make sure that you have good integration with existing lab equipment. The automated system should seamlessly integrate with other lab instruments and software, such as liquid handlers and data analysis tools, to streamline the workflow. Additionally, ensure the system has a user-friendly interface. A system with an intuitive interface and easy-to-use software can minimize training time and reduce the likelihood of user errors. Finally, consider your lab space and footprint of the system. A system that fits within your lab space and does not require excessive bench or storage space is ideal.

Q What are the barriers to entry?

KERKOW: There are two main barriers to entry when it comes to automation. The first is budget; evaluate the overhead setup costs to ensure they fit within your budget. Second, consider the time required for implementation. This includes ordering all necessary parts and materials. If the system involves numerous modules, this process can be time-consuming. Additionally, you need to validate the automation process. Once you have all the components, assess how long it will take to validate your workflow to ensure reliable results. This is particularly important for high-throughput applications.

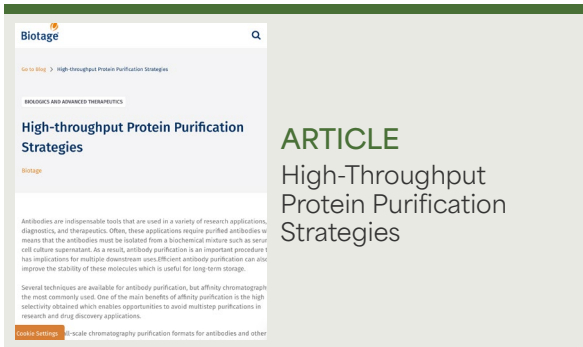
Q How do different platforms integrate with different lab workflows?

KERKOW: Spin columns require only a simple tabletop centrifuge. In contrast, filter plates need centrifuges, vacuum manifolds, gripper arms, and shakers, requiring the integration of multiple modules to complete the process. Magnetic beads need just a shaker and a magnetic module, but the quality of the magnetic module significantly impacts the results. The better the magnetic module you have, the better results you can expect. PhyTip columns are essentially pipette tips that can be placed directly onto your liquid handler, so no additional modules are required. They can be used just as any other pipette tip would be on your liquid handler.

PhyTip columns are essentially pipette tips that can be placed directly onto your liquid handler, so no additional modules are required. They can be used just as any other pipette tip would be on your liquid handler.

Q What is the user's experience automating the different platforms?

KERKOW: When automating different platforms, user experience varies significantly. A couple of key considerations are learning curve and processing time. PhyTip columns are very user-friendly and operate similarly to other pipette tips on a liquid handler. Process development is straightforward, involving simple parameter adjustments on the liquid handler, like flow rate and number of cycles. They are compatible with all major liquid handlers and come with pre-validated scripts. Spin columns, while easy to use, are not designed for automation and can be tedious for higher throughputs. However, they have a low learning curve.



Filter plates also have a low learning curve and are designed for higher throughput. However, they require many additional modules, which can make the validation process long and complex, and increases upfront cost. Finally, magnetic beads are compatible with automation. However, they are module dependent and can have a longer learning curve if you don't have

prior experience. Because it is not a resin-based platform for purification, this has its own nuances.

Processing time is the other consideration. This is the time required to run a purification protocol on each platform and is dependent on the efficiency of the binding kinetics. Because PhyTip columns use dual-flow chromatography, they are very efficient in binding samples to resin and can perform purification for a full plate in under an hour. Other platforms often require longer incubation times, resulting in a much longer protein purification process.

Q What are the risks associated with automation?

KERKOW: Automation in purification processes comes with several risks. Firstly, there is the risk of obtaining lower yields. To mitigate this, it's important to consider a purification platform with high-capacity resin to ensure higher yields to achieve target results. Secondly, protein aggregation is a concern. Mechanical stress during automation can cause proteins to aggregate, so selecting a platform that offers gentle purification is crucial. Lastly, to prevent cross-contamination, it's crucial to choose a platform that processes different samples in completely segregated environments, ensuring no possibility of transfer between them. For a system to be reliable and robust, preventing cross-contamination is essential.



How is dual-flow chromatography advantageous when automating?

KERKOW: Dual-flow chromatography is the process of slowly aspirating the sample through a packed resin bed, allowing for complete and maximum binding of the sample to the resin, and then a slow dispense, ensuring gentle purification and preventing aggregation of proteins.

Because PhyTip columns are essentially just pipette tips with a packed resin bed, they can be placed directly on a liquid handler deck, just as any other pipette tips would be. There are no additional modules required and no manual column preparation. You can simply adjust the liquid handling parameters, such as flow rate or cycle numbers, for process development.

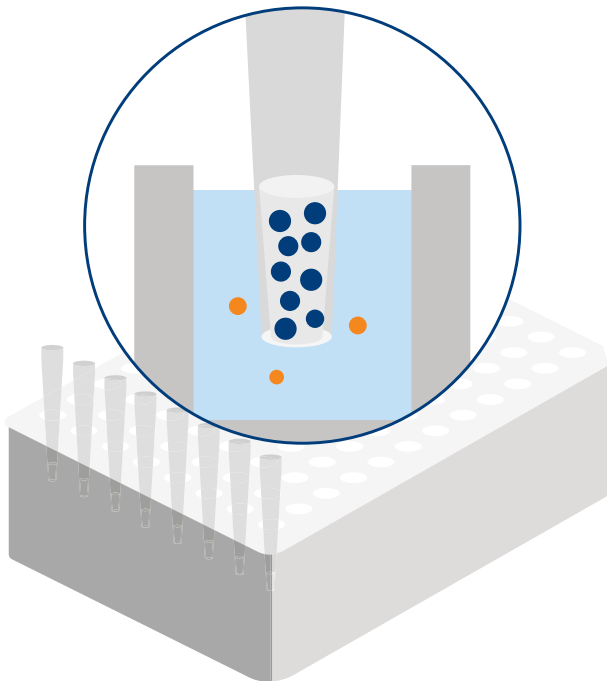
PhyTip columns are the optimal choice for biopharmaceutical industries seeking robust and efficient protein purification, especially when speed to outcome is crucial.

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PhyTip® Columns Versus Other Common Technologies:

A Comparative Analysis of Protein Purification Yields

This study focused on evaluating purified protein yields from PhyTip® columns against other common technologies, namely filter plates, spin columns and magnetic beads. PhyTip® columns containing ProPlus (MabSelect Sure) and Ni-IMAC resins were used to purify IgG spiked in CHO cells and His-eGFP in BL21 cells, respectively. Capacity testing showed that PhyTip® columns outperformed filter plates, spin columns, and magnetic beads, offering superior binding efficiency and higher yields across various protein concentrations. Besides yield, we have highlighted the ease of use, ease of automation, and process control benefits of PhyTip® columns, making them the product of choice for protein purification applications.

By: Gabrielle Kerkow; Shadie Nimri; Chris Suh, PhD; Farah Mavandadi, PhD



INTRODUCTION

Small-scale protein purification serves as a rapid and efficient method for screening samples for protein expression, stability, and functionality prior to scaling up to larger volumes, during both research and process development stages. This small-scale protein purification approach conserves samples by minimizing material usage and the purification process can be expedited through parallel high-throughput techniques. Common platforms that are well-suited for small-scale protein purification include PhyTip® columns, spin columns, filter plates, and magnetic beads.

The primary objective in screening labs is to isolate substantial quantities of protein for downstream analysis/assay. The main goal in process development revolves around optimizing purification methods and refining protocols to ensure reproducibility and facilitate seamless scalability for production. Various challenges that often impede protein purification efficiency in both screening and process development labs includes low protein yields, sample handling errors, equipment limitations, and restricted throughput. PhyTip® columns, designed with automation in mind, overcome these challenges and are rapidly gaining recognition as a key protein purification technique.

The unique Dual-Flow Chromatography (DFC) design of PhyTip® columns maximize sample binding to the resin and is optimal for high protein recovery. Through a series of sample and buffer aspiration and dispensing sequences directly through the resin-packed tip, DFC captures and releases biomolecules, offering a level of process control that is both automatable and reproducible.

This paper provides a comparison of how PhyTip® columns efficiently purify proteins relative to other commonly used protein purification platforms—namely filter plates, spin columns and magnetic beads. Yields were compared across purification platforms by saturating the resin to its dynamic binding capacity with protein.

MATERIALS USED

The comparative study was performed using Biotage 40 µL Ni-IMAC resin bed, 1 mL Hamilton tip (P/N: PTH-91-40-03); Cytiva Hi MultiTrap FF Ni Sepharose 6 Fast Flow Filter plate (P/N: 28400990); ThermoFisher Scientific Pierce™ Spin Columns Snap cap (P/N: 69725); ThermoFisher Scientific Pierce™ High Capacity Ni-IMAC MagBeads, EDTA compatible (P/N: A50591); Biotage 20 µL ProPlus resin bed, 1 mL Hamilton tip (P/N: PTH-91-20-07); Cytiva PreDictor MabSelect SuRe Filter Plate (P/N: 28925824); ThermoFisher Scientific Pierce™ High Capacity ProA MagBeads (P/N: A53036); Millipore Sigma IgG from Human Serum (P/N: 14506). All platforms

used Biotage standard IMAC buffer kit (P/N: BUF-01-00-03) and Biotage standard ProPlus buffer kit (P/N: BUF-01-00-01) for respective experiments.

METHODOLOGY

Ni-IMAC Resin With His-eGFP Samples

His-eGFP expressed in BL21 cells were purified with Ni-IMAC resin to test capacity across the four platforms, mentioned above. For each platform, four concentrations (1 mg/mL, 0.9 mg/mL, 0.6 mg/mL, and 0.3 mg/mL) of His-eGFP sample were purified in triplicate using 40 μ L of Ni-IMAC resin bed to determine capacity of each platform. Note for filter plates, 50 μ L of Ni-IMAC resin was used since Cytiva did not carry 40 μ L Ni-IMAC resin filter plates. The His-eGFP was diluted with 1x PBS. Each platform used 200 μ L of elution buffer. This was repeated twice for a total final volume of 600 μ L. Yield was measured using fluorescence spectroscopy of the expressed GFP on a ThermoFisher Scientific™ NanoDrop™ at 490nm wavelength. The NanoDrop™.

ProPlus Resin With IgG Samples

In order to mimic realistic samples used in antibody research labs, IgG from human serum proteins were spiked in CHO media and were tested for capacity against four platforms, mentioned above. For each platform, four concentrations (4 mg/mL, 3 mg/mL, 2.25 mg/mL, and 1.5 mg/mL) of human IgG sample were tested in triplicate on a ProPlus 20 μ L resin bed to determine the capacity of each platform. Each platform was eluted with 200 μ L of elution buffer. The eluted sample was analyzed using nanodrop measurement. This was repeated twice for a total final volume of 600 μ L elution.

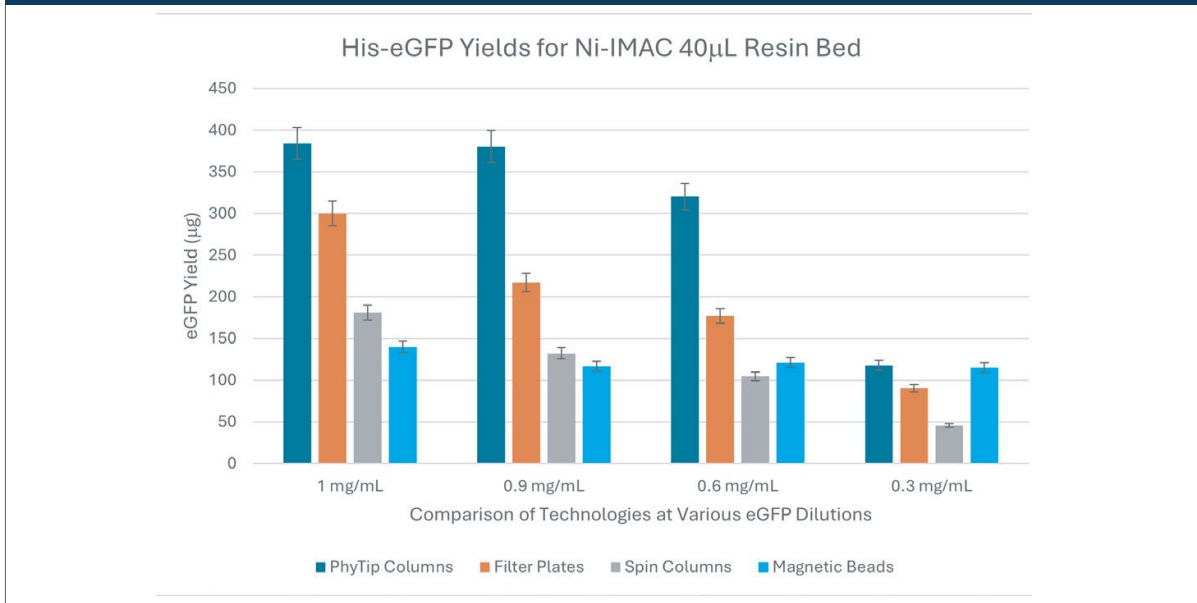
RESULTS AND DISCUSSION

Ni-IMAC Resin Testing

Significantly higher yields of His-eGFP protein were obtained using PhyTip® columns with Ni-IMAC resin compared to filter plates, spin columns, and magnetic beads. PhyTip® columns with 40 μ L Ni-IMAC resins performed significantly better than filter plates, spin columns, and magnetic beads for the three starting His-eGFP concentrations, 1 mg/ml, 0.9 mg/ml, and 0.6 mg/ml (**FIGURE 1**).

Many factors influence capacity including resin specificity, sample characteristics, and sample-to-resin interactions. The DFC mechanism of the PhyTip® columns maximizes binding efficiency and, in turn, maximizes the use of its resin capacity. Biologic samples such as recombinant proteins typically exhibit slow binding kinetics. Sufficient sample residency time on column is therefore crucial for efficient binding. This can be easily controlled for Phytips with DFC, by multiple pass-throughs of the sample through the column.¹ In comparison, the sample

FIGURE 1: The eGFP capacity testing on 40 μL of IMAC resin are an average of triplicates for each of the samples shown above. The dilutions were prepared with 1x PBS.



flow-through speed through spin columns or filter plates is very fast and difficult to control. This limits the time for sample to interact with the resin resulting in insufficient binding, and, hence, inefficient protein purification.

ProPlus Resin Testing

Significantly higher human IgG yields were achieved using PhyTip[®] columns with a 20 μL ProPlus resin bed as compared to filter plates, spin columns, or magnetic beads across a series of dilutions ranging 1.5 mg/ml to 4 mg/ml (**FIGURE 2**).

PhyTip[®] columns with ProPlus resin consistently outperformed other technologies, particularly at higher concentrations, showcasing their performance even at 4 mg/ml of IgG. These findings underscore the efficacy of PhyTip[®] columns in achieving higher protein yields across different concentrations, positioning them as a reliable choice for protein purification.

Biomolecules are easier to load on smaller resin-bed volumes. As the resin-bed volume size increases, there is typically a decrease in binding efficiency. However, PhyTip[®] columns offer better binding efficiency independent of resin bed volume. This is because DFC achieves prolonged and more complete perfusion of the resin bed, regardless of kinetic rate constants. The resulting separations are unaffected by column bed size, while additionally enabling fine control over separation parameters and allowing repeatable concentration of the sample as it passes through the resin.

FIGURE 2: Human IgG capacity testing on 20 μ L of ProPlus resin. Each sample seen is the average of triplicates for each dilution. The IgG protein sample is diluted and spiked with CHO media.

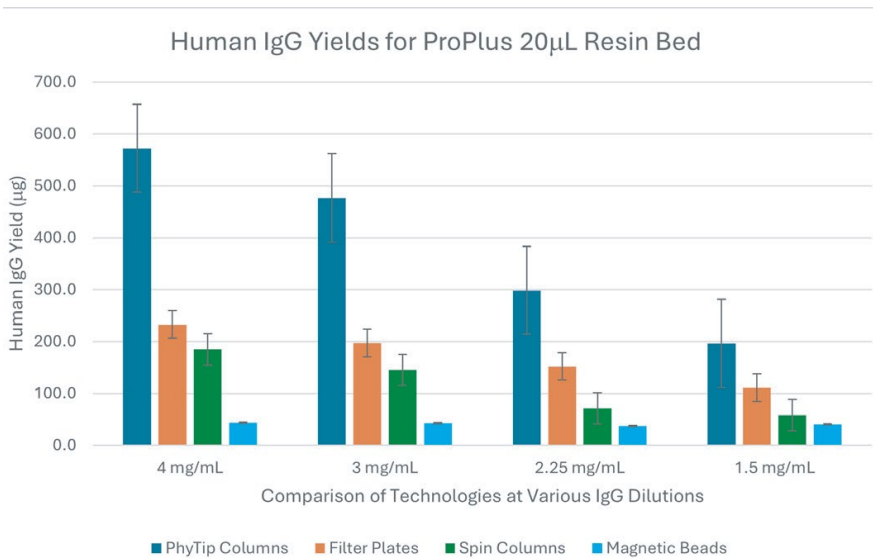
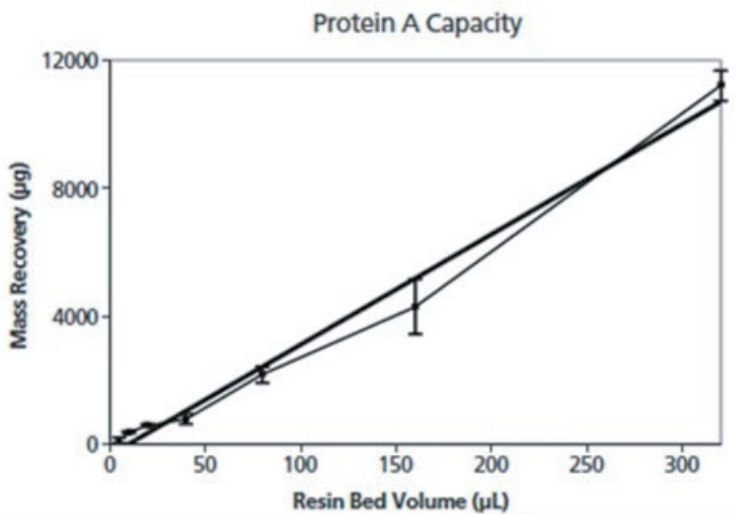


FIGURE 3: DFC efficiently binds protein to the specifications of the resin manufacturer. The yield accurately scales with resin bed volume. Experiments performed with 5- to 320- μ L bed volumes packing into a 1mL pipette tip column and agarose-based protein A affinity resin.¹



The scalability of PhyTip[®] columns is demonstrated in **FIGURE 3**, which shows a clear relationship between the volume of the resin bed and the amount of protein recovered. The study looked at the recovery of an IgG protein using columns with

FIGURE 4: 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.

		Mix Volume	Number of Mix Cycles	Aspirate Flow Rate	Dispense Flow Rate
Equilibration		200	4	Medium	Medium
Sample Capture	<input type="button" value="Aliquot Sample"/>	200	12	Medium	Medium
Wash 1		150	2	Medium	Medium
Wash 2		150	2	Medium	Medium
Wash 3		10	2	Medium	Medium
Elution	<input checked="" type="checkbox"/> Elution 2 (Disabled)	200	4	Medium	Medium
Sample Neutralization		50			

resin bed volumes ranging from 5 to 320 μ L and confirmed that the capacity of PhyTip® columns matched the specifications provided by the resin manufacturer under saturating conditions.¹

CONCLUSION

PhyTip® columns are specifically designed to automate and maximize binding efficiency and reduce overall sample processing time. While competitive technologies struggle to achieve higher yields with smaller resin bed volumes, PhyTip® columns do not have this limitation. The range of available resin bed sizes enables researchers to optimize and scale their purification protocols for highest yields and protein concentration.

SUPPLEMENTAL INFORMATION

Protein Purification Platform Protocols

Conditions were established according to the standard protocols for each platform. Although results may vary with optimization, the performance of each platform was evaluated based on the respective manufacturer's protocols.

PhyTip® Columns Protocol

This protocol followed the pre-generated automated script designed to run on liquid handlers. A cycle refers to one sequence of aspiration, pause, dispense and pause commands. Pre-generated script proceeds as follows: equilibration, sample capture, wash, elute.



Filter Plate Protocol

Followed manufacturer's standard protocol. Protocol steps included: equilibration (x3), sample capture, incubation (30 mins), wash (x3), elution.

Spin Columns Protocol

Followed manufacturer's standard protocol. The spin columns were filled with resin for respective ProPlus and IMAC testing. Protocol steps included: equilibration, sample capture, wash, elution. The spin columns were centrifuged for 15 seconds at 10,000 x g for each step.

Magnetic Beads Protocol

Followed Opentrons pre-generated automation standard script. Protocol steps included: equilibration, sample capture, incubation (30 minutes), wash (x2), elution.

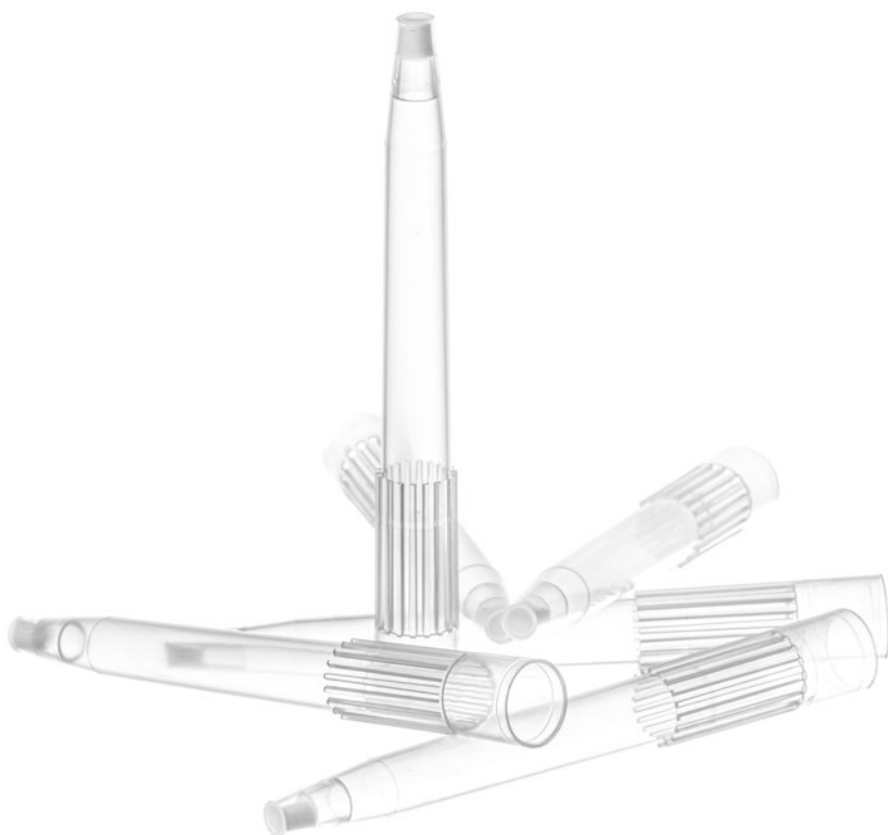
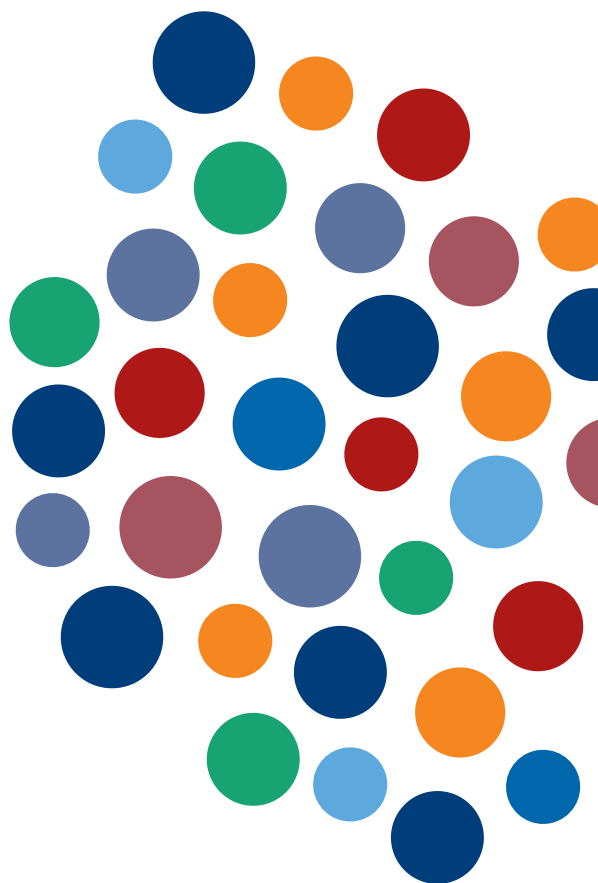
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PhyTip® Columns Versus Other Common Technologies:

A Comparative Analysis of Protein Purification Efficiency and Yield Consistency

PhyTip® columns were evaluated against other commonly used purification platforms for purifying recombinant proteins. The study assessed performance quality by examining yield consistency and protein purity using Ni-IMAC resin in PhyTip® columns, filter plates, magnetic beads and spin columns. PhyTip® columns outperformed filter plates, spin columns, and magnetic beads, providing higher yields with lower variability. The fully automated purification process ensured consistent recovery and batch-to-batch reproducibility. Additionally, the slow flow rates in PhyTip® columns prevented protein denaturation and aggregation, enabling efficient purification of small volume and low-titer samples.

By: Gabrielle Kerkow; Shadie Nimri; Chris Suh, PhD; Farah Mavandadi, PhD



Automation plays a crucial role in small-scale purification during early-stage drug discovery and research. By efficiently processing a large number of protein samples in small volumes, researchers can gain valuable insights into protein expression, stability, and functionality.

Traditional protein purification platforms, including filter plates, spin columns, and magnetic beads encounter issues with protein aggregation and cross-contamination. These challenges often lead to inconsistent recovery and reproducibility, particularly when handling low-titer samples. PhyTip® columns, which operate at slow flow rates, prevent protein denaturation or aggregation, making them ideal for small volume and low-titer samples. The ability to fully automate the purification process with PhyTip® columns ensures consistent recovery and batch-to-batch reproducibility.

In high-throughput labs, maintaining consistent yield and purification efficiency is crucial for ensuring quality results. This study evaluates yield consistency by analyzing 48 samples processed under standard conditions. Additionally, purification efficiency is assessed by comparing eluted samples from the four different platforms using SDS-PAGE gel electrophoresis.

MATERIALS USED

The comparative study was performed using Biotage 40 µL Ni-IMAC resin bed, 1 mL Hamilton tip (P/N: PTH-91-40-03); Cytiva Hi MultiTrap FF Ni Sepharose 6 Fast Flow Filter plate (P/N: 28400990); ThermoFisher Scientific Pierce™ Spin Columns Snap cap (P/N: 69725); ThermoFisher Scientific Pierce™ High-Capacity Ni-IMAC MagBeads, EDTA compatible (P/N: A50591); All platforms used Biotage standard IMAC buffer kit (P/N: BUF-01-00-03) and Biotage standard ProPlus buffer kit (P/N: BUF-01-00-01) for respective experiments.

METHODOLOGY

Standard protocol conditions were followed for each platform. While optimization may yield different outcomes, in this study the performance of each platform was evaluated according to the respective manufacturer's protocol. Four commonly used protein purification platforms were evaluated, each platform processed 300 µg of His-eGFP using 20 µL of Ni-IMAC resin. For the filter plate 25 µL of Ni-IMAC resin was used as 20 µL resin bed filter plate was not commercially available.

For assessing yield consistency, the percentage recovery and coefficient of variation (CV) values were compared for 48 samples processed on each protein purification platform. Yield was measured using fluorescence spectroscopy of the expressed GFP on a ThermoFisher Scientific™ NanoDrop™ at 490 nm wavelength.

For assessing purification efficiency, samples purified using PhyTip® columns were compared with those purified using filter plates, magnetic beads and spin columns via SDS-PAGE gel. Additionally, the imidazole wash efficiency was evaluated using a wash buffer gradient. Each platform was tested in duplicate for accuracy.

RESULTS AND DISCUSSION

Yield consistency and purification efficiency for His-eGFP samples were compared using different purification platforms: PhyTip® columns, filter plates, magnetic beads and spin columns containing Ni-IMAC resin. The goal of these experiments was to assess the performance of each protein purification platform in terms of protein yield, result reproducibility and purified protein quality.

Among the four platforms that were evaluated, PhyTip® columns provided the highest yield, averaging 216.60 µg with 72.2% recovery, with low sample-to-sample variation (**FIGURE 1, TABLE 1**). In comparison, filter plates providing the second highest yields and recovery (189.21 µg; 60.45%), followed by magnetic beads (119 µg; 39.56%) and spin columns (103.86 µg; 34.53%). Notably, PhyTip® columns demonstrated the lowest sample-to-sample variability (CV<10%) and the highest consistency (**TABLE 1**) all while requiring less elution buffer than the competitive technologies resulting in higher protein concentrations.

The unique design of the PhyTip® columns incorporates a thin hydrophilic frit surrounding the packed resin bed, resulting in virtually no dead volume. A very

FIGURE 1: 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.

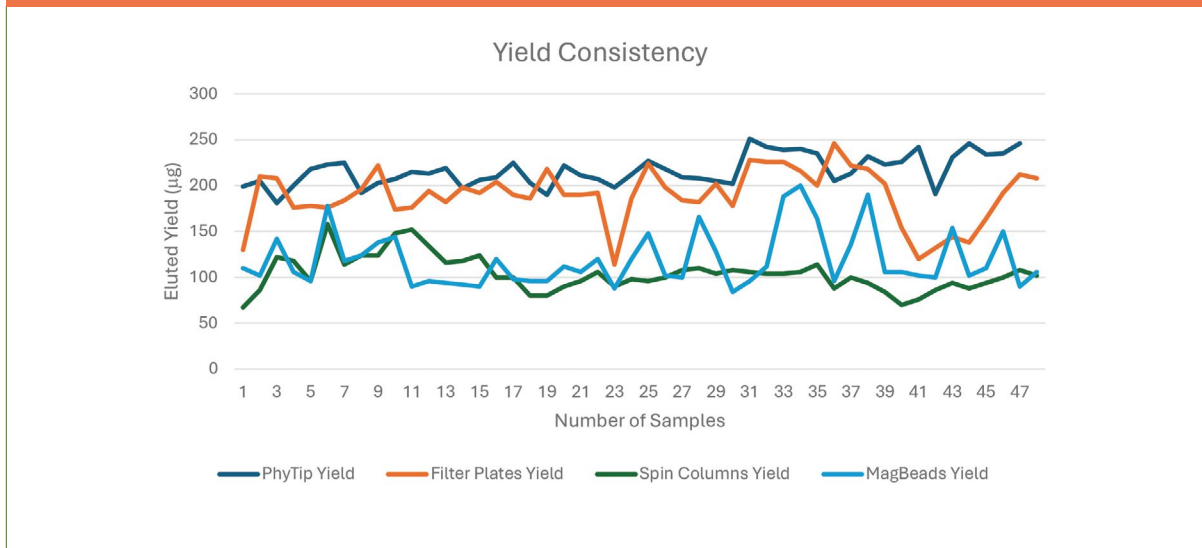


TABLE 1: Consistency experiment calculations.

	PHYTIP* COLUMNS	FILTER PLATES	SPIN COLUMNS	MAGNETIC BEADS
Average Yields	216.60	189.21	103.86	119.00
Standard Deviation	16.97	29.42	19.25	29.78
Coefficient of Variation	7.83%	15.55%	18.54%	25.02%
Percent Recovery	72.20%	60.45%	34.53%	39.56%

small amount of liquid (sample or buffer) aspirated through the hydrophilic frit efficiently covers the entire resin bed. As a result, less elution buffer is required. For example, a 40 µL resin bed volume in a PhyTip® column can be effectively eluted with 120 µL elution buffer, whereas other platforms typically require 200 µL of elution buffer. This efficient elution process concentrates the final sample while minimizing contamination compared to other methods (see SDS-PAGE gel image in **FIGURE 2**). This phenomenon is known as the ‘tip concentrating effect’ of PhyTip® columns.

FIGURE 2: 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.



The improved performance of PhyTip® columns can be attributed to their Dual Flow Chromatography (DFC) mechanism. Unlike other protein purification platforms, the unique processing method in these columns maximizes interaction time with the resin. This enhances sample binding efficiency, ensures consistent yield, reduces sample-to-sample variation and enhances chromatographic selectivity, resulting in the desired separations.¹

EVALUATING TECHNOLOGY IMPACT ON CONSISTENCY ACROSS FOUR PLATFORMS

Liquid handling robots regulate sample flow through the PhyTip® column resin bed, minimizing sample-to-sample variation. Residence time for binding, washing, and elution of the target protein from the resin can be optimized by adjusting parameters like capture cycles and flow rates. Slow flow rates prevent protein denaturation and

FIGURE 3: SDS-PAGE gel image of PhyTip® column imidazole efficiency testing. Lanes left to right: Ladder, Fraction1, Fraction 2, Fraction 3, Fraction 4, Fraction 5, Ladder. Fractions are referring to concentrations of imidazole from TABLE 1.

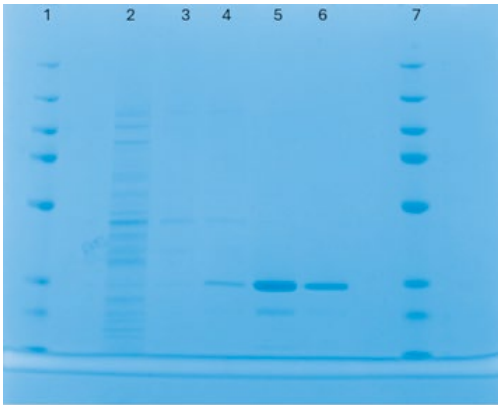
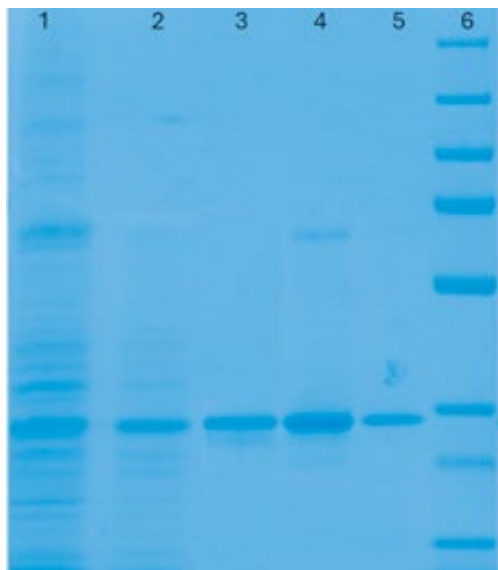


FIGURE 4: SDS-PAGE gel image of filter plate imidazole efficiency testing. Lanes left to right:

Fraction1, Fraction 2, Fraction 3, Fraction 4, Fraction 5, Ladder. Fractions are referring to concentrations of imidazole from TABLE 1.



aggregation, making this technique ideal for small volume and low-titer samples. Additionally, slow flow rates preserve protein structure and functionality, resulting in highly functional proteins suitable for downstream applications. SDS-PAGE gel analysis of imidazole wash buffer fractions at various concentrations demonstrates that PhyTip® columns effectively remove contaminants while maintaining the integrity of His-eGFP protein (**FIGURE 3**).

For **FIGURE 3** and **FIGURE 4**, Lanes 2-6 are separate fractions of sample eluted with various concentrations of imidazole. Fraction one is 10 mM imidazole concentration, fraction two is 20 mM imidazole concentration, fraction three is 60 mM imidazole concentration, fraction four is 200 mM imidazole concentration, and fraction five is 250 mM concentration.

Filter plates, on the other hand, rely on less precise external factors such as a vacuum pressure. Variations across different well positions significantly impact recovery and sample purity based on their location on the plate (**FIGURE 3 and 4**) this is demonstrated by the sharper band seen in lane 5 (**FIGURE 3**).

The performance magnetic beads relies heavily on their interaction with the magnetic module. This interaction can cause bead aggregation or sample/ buffer carry-over, affecting purification consistency and yields. Demagnetized beads often become a source of impurity that is problematic in downstream analysis, particularly with mass

FIGURE 5: 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.

The screenshot displays the 'Protein Purification' software interface. At the top, it shows 'Resin' set to 'Protein A/Protein G', 'PhyTip and Resin Volume' set to '1mL, 20uL', and 'Number of Samples' set to '12'. Below this, there are several rows of parameters, each with a toggle switch on the left and numerical or flow rate inputs on the right. The parameters are: Equilibration (toggle on), Sample Capture (toggle on, with an 'Aliquot Sample' button), Wash 1 (toggle on), Wash 2 (toggle on), Wash 3 (toggle off), Elution (toggle on, with 'Elution 2 (Disabled)' toggle off), and Sample Neutralization (toggle on). The numerical inputs are: Mix Volume (200), Number of Mix Cycles (4), Aspirate Flow Rate (Medium), and Dispense Flow Rate (Medium) for Equilibration; Mix Volume (200), Number of Mix Cycles (12), Aspirate Flow Rate (Medium), and Dispense Flow Rate (Medium) for Sample Capture; Mix Volume (150), Number of Mix Cycles (2), Aspirate Flow Rate (Medium), and Dispense Flow Rate (Medium) for Wash 1; Mix Volume (150), Number of Mix Cycles (2), Aspirate Flow Rate (Medium), and Dispense Flow Rate (Medium) for Wash 2; Mix Volume (10), Number of Mix Cycles (2), Aspirate Flow Rate (Medium), and Dispense Flow Rate (Medium) for Wash 3; Mix Volume (200), Number of Mix Cycles (4), Aspirate Flow Rate (Medium), and Dispense Flow Rate (Medium) for Elution; and Mix Volume (50) for Sample Neutralization. At the bottom, there are 'Cancel' and 'Start' buttons, and the Biotage logo.

Step	Mix Volume	Number of Mix Cycles	Aspirate Flow Rate	Dispense Flow Rate
Equilibration	200	4	Medium	Medium
Sample Capture	200	12	Medium	Medium
Wash 1	150	2	Medium	Medium
Wash 2	150	2	Medium	Medium
Wash 3	10	2	Medium	Medium
Elution	200	4	Medium	Medium
Sample Neutralization	50			

spectrometry. These platform-specific considerations underscore the importance of choosing the right technique to achieve consistent and high-quality protein purification outcomes.

CONCLUSION

PhyTip® columns offer distinct advantages over other purification platforms, such as filter plates, spin columns, and magnetic beads. Having a 96-well format is ideal for parallel, multivariate method development. Users can fine-tune parameters affecting yield, purity, and protein activity/stability in a single run. The slow flow rates prevent protein denaturation and aggregation, making PhyTip® columns particularly suitable for low-titer and low volume sample screening. Additionally, the ability to fully automate the purification process using liquid handling robotics ensures consistent recovery and batch-to-batch reproducibility. Overall, PhyTip® columns outperformed all other technologies on the evaluation criteria of exceptional purity, highest yield and most consistent reproducibility. These columns are the optimal choice for biopharmaceutical industries seeking robust and efficient protein purification, especially when speed-to-outcome is crucial.

SUPPLEMENTARY MATERIAL

Protein Purification Platform Protocols

PhyTip® columns protocol: This protocol followed the pre-generated automated script designed to run on liquid handlers. A cycle refers to one sequence of aspiration, pause, dispense and pause commands. Pre-generated script proceeds as follows: equilibration, sample capture, wash, elute.

Filter Plate Protocol

Followed manufacturer's standard protocol. Protocol steps included: equilibration (x3), sample capture, incubation (30 mins), wash (x3), elution.

Spin Columns Protocol

Followed manufacturer's standard protocol. The spin columns were filled with resin for respective ProPlus and IMAC resin testing. Protocol steps included: equilibration, sample capture, wash, elution. The spin columns were centrifuged for 15 seconds at 10,000 x g for each step.

Magnetic Beads Protocol

Followed Opentrons pre-generated automation standard script. Protocol steps included: equilibration, sample capture, incubation (30 minutes), wash (x2), elution.

REFERENCE:

1. <https://www.chromatographyonline.com/view/dual-flow-chromatography>