

Automated Workflow with Hamilton and PhyNexus

Introduction

[PhyNexus](#) provides a high-performance system for small scale protein separations to life science researchers worldwide. PhyNexus' flexible technology enables optimized preparation of 1-96 samples at a time using Hamilton Nimbus and Star systems that provide fully walk-away and scalable operation.

[PhyNexus](#)' patented [Dual Flow Chromatography](#) (DFC) tip technology for proteins and plasmid purification offers the most efficient and cost effective way to purify recombinant proteins, antibodies, and plasmids. It allows bidirectional flow of the sample and buffers through the column to maximize the use of the resin by increasing capture and elution of the target protein, leading to the highest yield possible.

High Throughput Affinity Purification

Large culture volumes are grown in order to achieve the sufficient expression of antibodies from transient transfection. The supernatant from each culture is then collected in a 50 mL conical tube and loaded in the Hamilton Star. From there, automation takes over.

Hamilton's CORE 1000 μ L Pipetting Channel with variable spanning capabilities then transfers 1 mL from each conical to a corresponding well of a 96-deep well plate. Next, Hamilton's CO-RE 96 MPH head engages 96 1 mL PhyTip columns to purify the sample. The process is repeated through automation until the lab generates the amount of purified antibody desired from 1 mL to 50 mL or more.



Spin down and load



Aliquoting 1ml in 96 well plate



PhyNexus mAB purification

The production of recombinant proteins has made a revolutionary impact on human healthcare by enabling mass production of safe and effective therapeutic drugs such as hormones, growth factors, blood coagulation products, thrombolytic agents, vaccines, and monoclonal antibodies (mAbs).

Hamilton and [PhyNexus](#) have teamed up to deliver an automated workflow solution which purifies up to 50 mL volumes of target antibody from transient transfection. Purifying sufficient antibody volumes for downstream applications can be a tedious and time-consuming task. Low expression efficiency of recombinant proteins from transient transfection means microscale purification must be repeated many times, in order to yield the sufficient concentrated solution of antibodies.

- Entire purification in less than 15min
- Very high protein yield and concentration with excellent purity
- Flexible choice of resin bed size



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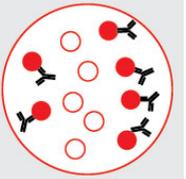
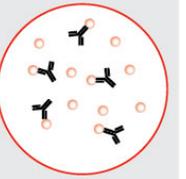
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PhyNexus PhyTip Columns

[PhyTip columns](#) are ready-to-use patented pipette tip columns with the resin bed pre-packed between two low dead volume frits in the tip of the column. [PhyTip columns](#) deliver superior purification results through the use of [Dual Flow Chromatography](#), a gentle back-and-forth flow over the ligand that drives the binding reaction to full equilibrium. PhyTip columns deliver the highest yields and concentrations of pure proteins without harsh spinning or shearing effects that can alter proteins and protein complexes.

PhyNexus

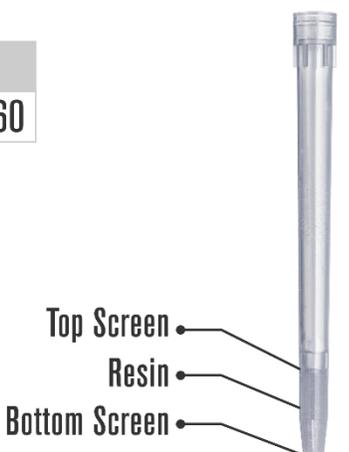
**DUAL FLOW
CHROMATOGRAPHY**

| | PhyTip Columns | Spin Columns | Magnetic Beads |
|-----------------------------|--|---|---|
| Binding(capture) Efficiency |  |  |  |
| Complete Loading | ✓ | ✗ | ✗ |
| High Concentration | ✓ | ✗ | ✗ |
| Any Sample Size | ✓ | ✗ | ✗ |
| Any Column Size | ✓ | ✗ | ✗ |
| Scalable | ✓ | ✗ | ✗ |

PhyTip Column Resin Bed Size Options and Benefits

[PhyTip columns](#) come in a variety of resin bed sizes, offering researchers great flexibility to optimize antibody recovery against anti- body concentration. A larger resin bed size provides higher capacity antibody binding, and therefore greater antibody recovery. This can be very beneficial when working with the low expression efficiency of antibodies from transient transfection. However, larger resin beds require larger elution volumes, resulting in greater dilution. The researcher can choose the size that delivers the optimal balance of capture and concentration. With a range of resin bed volumes, resins types, and single use convenience, most applications using PhyTip columns on the Hamilton do not require additional accessories beyond just the deck and liquid handling head.

| Resin Volume (µL) | | | | | |
|-------------------|------|----|----|----|-----|
| 5 | 10 | 20 | 40 | 80 | 160 |
| Tip Volume (µL) | | | | | |
| 300 | 1000 | | | | |
| QTY/Box | | | | | |
| 96 | | | | | |



[PhyNexus](#) maintains a strong collaborative relationship with Hamilton applications specialists to guide customers through the setup process, providing support for deck set up, programming, chemistry and troubleshooting to ensure proper performance of the runs.

PhyTip Columns for Other Applications on the Hamilton

[PhyNexus](#) provides a host of other solutions, all compatible with the Hamilton STAR and Nimbus platforms, delivering 96-channel, high throughput solutions, including:

- Affinity pull-down purifications (His-tagged proteins, GST-tagged proteins, biotin-tagged proteins)
- Plasmid DNA purification
- Ion Exchange Chromatography (weak & strong cation, weak & strong anion)
- HTP Gel filtration for desalting and buffer exchange (fully automated)
- HTP Mass spec protein & peptide clean-up (C18, C4, gel filtration)

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Hamilton STAR Ideal for High Volume, High Throughput Antibody Purification

The Hamilton Star (and STARlet, and STARplus) is uniquely suited for high throughput, automated purification of antibodies produced in transient transfection. This includes the ability to transfer and redistribute sample volumes from various formats, as well as process sufficient volumes to generate larger amounts of target in reasonable times.

CO-RE Variable Spanning, 8-channel Pipetting Head

Simply stack up to 96 conical tubes with 96 different samples in the Hamilton STAR, and the unique, variable spanning, 8-channel pipetting head can draw 1000 μ L from each conical tube and deliver the sample to a 96-well plate. Moreover, the STAR can perform this in parallel, while the 96-channel head is performing capture, wash, and elution on another plate.

1000 μ L Volume Capacity CO-RE 96 Pipetting Head

The Hamilton Star is one of the only advanced liquid handling instruments capable of processing up to 1000 μ L volumes per channel on a 96-channel head. At these volumes, [PhyTip columns](#) can quickly process 50 mL of target samples (times 96 samples) with only 50 passes. The Hamilton STAR makes processing large volumes both quick and easy.

Collaborative Software and Methods Ready for Customer Applications

Hamilton and [PhyNexus](#) have worked together (in Europe and United States) to optimize Hamilton's Venus software to perform the methods required for optimal protein capture, wash and elution, using PhyTip columns. PhyNexus combines the ease of automation with the highest protein yields, concentrations and quality using [Dual Flow Chromatography](#). Hamilton's engineers are experts in helping to implement required software methods for volumes, cycles, and timing.

Industry-Leading Support

With both Hamilton and [PhyNexus](#), you get the very best in customer service and support to ensure your purifications are fast and easy. Whether it is help with hardware, software, or purification methods, both companies have a long reputation of excellent customer service. Together, we will get you the help you need and we are ready to find new applications for your Hamilton instrument with PhyTip columns.



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