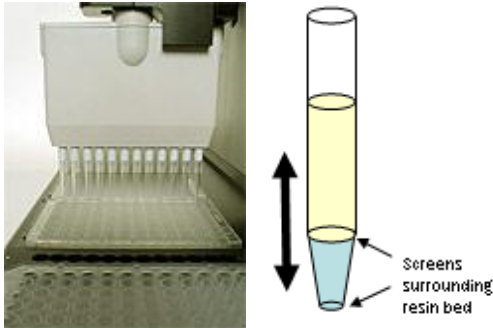


Automated Protein Purification or Plasmid DNA Workflow with PhyTip® Chromatography Tip Technology

PhyNexus has patented chromatography tip technology for high throughput affinity chromatography for proteins and plasmid purification. PhyNexus' PhyTip® column's fully automated technology enables purification workflow for:

- Chromatography separations based on pipette tip columns
- 1-12 or 1-96 samples processed in parallel with no performance compromise
- Superior concentration, purity and activity of recovered protein or plasmid
- Compatible with all major robotic platform operation with no modifications
- Complete walk-away automation
- Pure samples in as little as 15 minutes



PhyTip columns are the most efficient and cost effective way to purify recombinant proteins and antibodies using standard affinity resins. Their unique design allows bidirectional flow (see arrow in figure) of the sample 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. The dead volume is minimized with low surface area, thickness and pore

volume of frits. The design of the screens surrounding the resin allows elution volumes as low as 10 μ L, and therefore high concentration product. The high efficiency bidirectional washes lead to the highest possible sample purity. Affinity purification can be performed with any resin of choice. Higher performance is obtained than with any other resin purification method.

Sample Separation

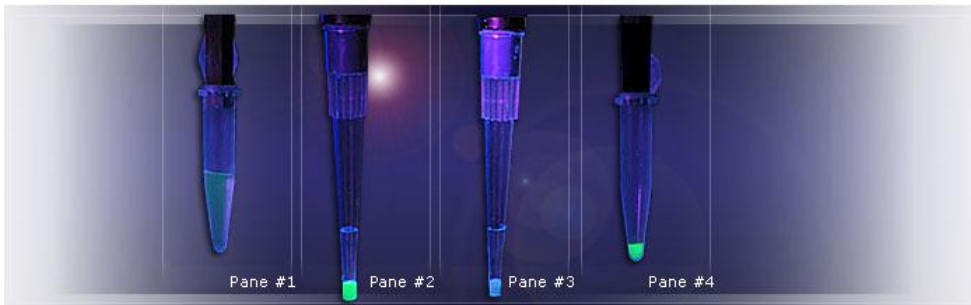


Figure 2. Affinity purification of antibody sample ... Capture, Purify, Enrich

Pane#1. Low concentration Alexa488-labeled antibody showing very little fluorescence.

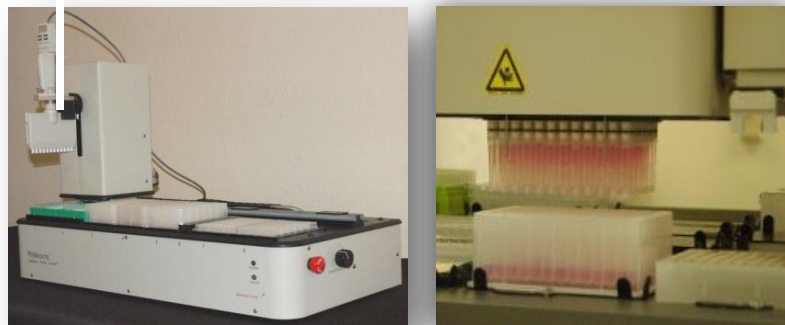
Pane#2. Protein A PhyTip column after processing antibody sample. Alexa488-labeled antibody has been successfully *captured* on the PhyTip column. The sample is *purified* by washing the column.

Pane#3. PhyTip column after the elution step; there is little discernible fluorescence due to the very high yield for the separation.

Pane#4. High *enrichment* of the Alexa488-labeled antibody is achieved after elution.

1-12 and 1-96 parallel processing:

Patented PhyTip® column technology is a unique walk away bench-top separation process controlled with a high level of precision for life science researchers who prepare protein samples or plasmid DNA for analysis. By utilizing industry standard resins, it allows for the unattended parallel processing of a range of samples and volumes providing the highest flexibility in meeting the needs of a diversity of applications. Columns can be used to process sample volumes from 10mL to 25ml (sample volumes exceeding the tip column volume are processed as a series of fractions during the capture step).



Superior concentration, purity and activity of recovered protein or plasmid:

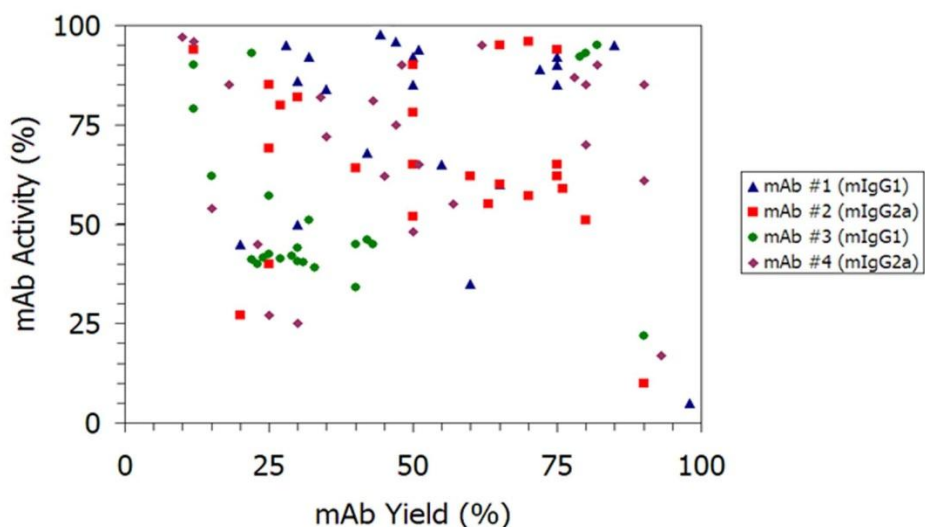
PhyTip columns employ a Virtual Heart-Cut Elution™ process to produce the highest possible concentration and purity of product. In conventional affinity chromatography, the center of the eluted peak (heart cut of the peak) is captured to produce the highest concentrated product at the highest possible purity. In Virtual Heart-Cut Elution™ technology offered by PhyNexus, the affinity resin in the column is fully loaded. Back and forth flow of sample through the pipette tip column enables the resin to be fully loaded and complete. As many sample aliquots as desired can be loaded.

In the next step, thin, low dead volume and low surface area column frits allow complete washing of the resin bed with a very high wash volume to resin volume ratio (up to 50X) to thoroughly remove non specific bound contaminants.

Finally, fine control of the elution volume allows very small and complete elution of the purified protein producing the highest possible concentrations. Because the column is so highly loaded and the elution volume is small, the Virtual Heart-Cut Elution process produces a product as if the heart of a chromatography peak is recovered. This process produces concentrations that are 5-10 times higher than competing technologies. For 5 µL bed columns, elution volumes as low as 10 µL are possible. Finally, the

separations can be easily run in parallel to develop the absolute superior conditions for purity, yield and concentration.

Many customers also use ELISA to test for protein activity. High protein recovery is useless unless the protein that is recovered has retained activity.



Four different antibody clones were screened for optimal purification conditions by varying 24 different buffer variables using PhyTip columns on a 96-channel robot. Yield was measured by ELISA and activity was measured by SPR. In this way, purification conditions were screened and discovered that achieved both high recovery and high activity.

PhyTip columns compatible with all major robotic platforms:

The PhyNexus MEA Purification Systems allow for processing of 1-12 samples at a time. The columns are also manufactured for 96 channel liquid handling robots include Tecan, Caliper, Dynamic Devices, Perkin Elmer, Hamilton, Velocity 11, and Beckman.