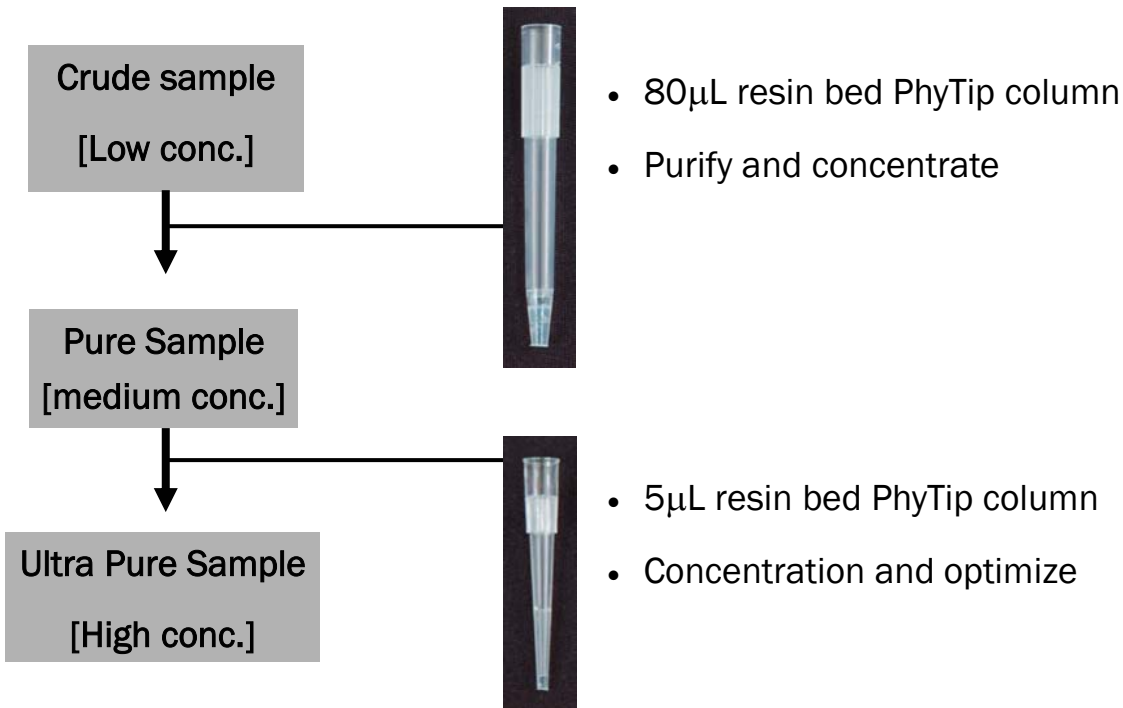


## THE PHYNEXUS PHYTIP® COLUMN METHOD FOR OBTAINING HIGH CONCENTRATIONS OF ACTIVE PROTEINS

A common problem in the purification of proteins expressed at low concentration is a resulting pure protein that is too dilute for subsequent assays. Traditional methods for concentration of proteins utilize protein precipitation steps, centrifugal filter devices, or performing a hard-cut elution on an FPLC. Though the first two methods are widely accepted, the poor reproducibility, low efficiency recovery, loss of activity, and inability to automate requires that new tools be developed. Performing a hard-cut has the disadvantage of requiring careful control of gradients and timing the hard cut with a fraction collector. And even then, the tightness of the peak is dependent upon the column separation conditions.

The PhyNexus Two-Step Purification Method is centered upon unparalleled performance from small resin bed volume columns. In the first step, large bed volume PhyTip columns purify protein from enough cell lysate or supernatant in order to obtain a desirable yield and purity, while affording a sizable increase in concentration. In the second step, small PhyTip columns containing as little as 5 $\mu$ L of resin is used to recapture the pure sample. The small resin bed column is loaded to the maximum capacity that the protein concentration will allow and a small volume elution, 10-15 $\mu$ L, results in the highest concentration for any commercially available affinity purification system. The efficiency of elution from a PhyTip column combined with retention of protein activity provide the best solution for an automatable concentration process.

- Maintain protein activity
- Perform purification and concentration in a simple, two-step method
- Automate the methods for walk-away convenience
- Optimize buffer types and concentration for preserving protein structure
- Achieve the highest possible protein concentration possible while maintaining activity



## THE PHYNEXUS PHYTIP® COLUMN CONCENTRATION METHOD CONT'D

**Table 1: Purification of Fc-fusion proteins using the two-step PhyNexus concentration method**

	Total protein recovered (µg)	Volume recovered (µL)	Concentration (µg/mL)	Fold concentration increase
80µL PhyTip ProPlus purification	4.9	300	16	>10
5µL PhyTip ProPlus concentration	2.9	14	215	>200

10mL PBS samples spiked with Fc-fusion protein standard (R&D Systems, 342-CD) to a final concentration of 1µg/mL was purified with 80µL PhyTip ProPlus columns (PTR 91-80-07) and eluted in 240µL low pH buffer followed by neutralization with 60µL high pH buffer. A small aliquot was analyzed by quantitative HPLC and the remaining sample was subjected to concentration by 5µL PhyTip ProPlus columns (PTR 92-05-07) using 10µL elutions followed by 2.5µL neutralization.

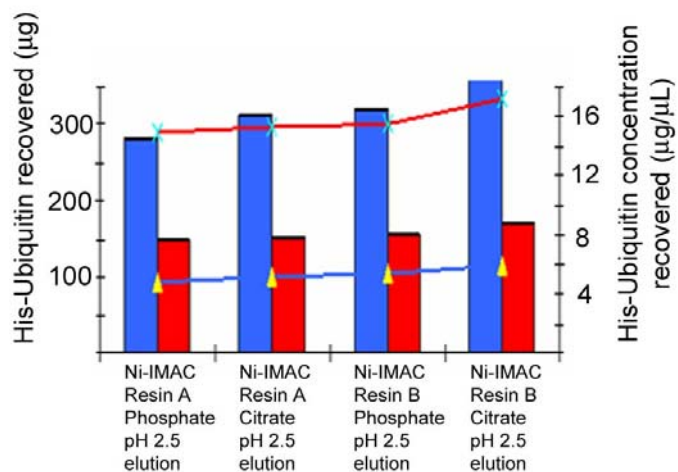


Figure 1: His-tagged ubiquitin (Boston Biochem, U-530) was spiked into PBS and processed by PhyTip columns containing 20µL of either of two types of Ni-IMAC resins. Samples were eluted with 60µL of either low pH phosphate or citrate elution buffers and either quantified or concentrated using 5µL PhyTip columns and 15µL of 500mM imidazole for elution. The histogram shows the recovery of sample before concentration (blue bars) or following concentration (red bars). And the blue and red lines depict the concentration of His-Ub before and after concentration, respectively. Though the concentration step results in loss of His-Ub, a 3 to 4 fold increase in concentration is obtained.

PhyTip columns are specifically manufactured for the automated MEA bench top, walk-away system.



PhyTip columns can also be used with the ME 200 and ME 1000 semi-automated purification systems.

