

PHYTIP® COLUMNS: BEYOND AFFINITY PURIFICATION Adapting ion exchange to small-scale columns

PhyNexus' family of PhyTip products includes the industry proven ProA, ProG and ProPlus Columns for antibody purification, IMAC Columns for His-tagged protein purification, Glutathione Columns for GST-tagged protein purification and Streptavidin Columns for biotin-tagged protein and nucleic acid purifications. PhyNexus has taken the core PhyTip technology beyond affinity purifications to develop Gel Filtration Columns as well as expand upon the custom resin packing service. The newest separation chemistry to be adapted into the PhyTip column is ion exchange. In traditional ion exchange chromatography, samples, wash buffers and gradient elution buffers pass through packed columns in one direction. The PhyTip column is able to simulate this chromatographic separation in a small, pipette tip column by driving the binding and release steps to equilibrium. This is not achieved by using high back-pressure, large columns, but instead by PhyNexus' patent pending back-and-forth flow.

- Develop ion exchange purification methods in a fast, efficient manner
- Automation compatible on the MEA Personal Purification System or other robotic systems
- Take advantage of parallel processing to screen resins
- Applications include conditions optimization, imidazole removal aftr His-tagged protein purification and high-throughput replacement of finishing columns



Recovery of BSA using a weak anion exchange resin (left) and lysozyme using a weak cation exchange resin (right). A matrix of binding buffers of varying pH and elution buffers of increasing salt concentration allow for rapid process optimization using PhyTip columns.



Further process optimization of lysozyme binding conditions with cation exchange columns: binding efficiency as a function of residence time and capture cycles at pH 6.0.



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Perform resin screening in for most efficient capture of target proteins

Table 1: Ion exchange resins used for	
screening binding efficiency	
Resin	Туре
А	Weak cation exchange
В	Strong cation exchange
С	Strong cation exchange
D	Strong cation exchange
Е	Strong cation exchange
F	Strong anion exchange
G	Strong anion exchange
Н	Weak anion exchange

5µL of each of eight different ion exchange resins (Table 1) were packed into PhyTip columns and tested in parallel for efficiency of capture. Using the MEA Personal Purification System the columns were equilibrated in 100µL of 25mM Tris pH 8.0, 5mM NaCl using 1 cycle at 0.25mL/minute, and performed 8 capture cycles at 0.25mL/minute of 200µL capture buffer spiked with BSA to 2mg/mL. Starting sample and sample flow through was quantified by the NanoDrop UV spectrometer for absorbance at 280nm. The lysozyme capture efficiency was performed in the same way except columns were equilibrated in 25mM Na-citrate, pH 6.0, 5mM NaCl buffer and samples were composed of the same equilibration buffer spiked with lysozyme to 0.4mg/mL. Comparison of capture of two proteins using 8 different resins took less

PhyTip ion exchange columns available as a custom product, for ordering details please contact PhyNexus

Columns are available in the following sizes:

200 μ L column with ion exchange resin volume of 5 μ L and 20 μ L

1000 µL column with Pro Plus resin volume of 10, 20, 40, 80, 160, and 320µL

