Micro-scale Protein Enrichment Using Novel Affinity Column Technology

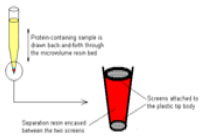
Jeremy Lambert, Utpal Banik, Christopher Hanna - **PhyNexus, Inc., San Jose, CA**

Abstract

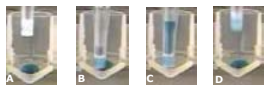
Traditional protein purification methodologies are sufficient for bulk sample preparation but cannot readily be used for preparing high concentrations of pure protein in small volumes often needed for high sensitivity protein analyses. In order to overcome this bottleneck, we have developed a unique micro-volume enrichment technology. PhyTip™ columns utilize low volume affinity resin beds that specifically bind affinity tags (e.g. His-tagged proteins) or antibodies (e.g. IgG). Here we present this novel miniaturized sample preparation technology that can rapidly purify and enrich antibody or affinity tagged proteins at micro-volume scale.

PhyTip™ Columns

PhyNexus has developed a unique, yet simple process to purify and enrich antibodies or recombinant proteins containing affinity tags. To demonstrate the effectiveness of this new sample preparation system, antibodies and recombinant affinity-tagged proteins were processed with PhyTip™ columns. These columns are specifically designed to contain micro-volume quantities of conventional affinity separation media in a manner that introduces virtually zero dead volume .



PhyTip™ columns encapsulate a small amount of resin within the end of a pipette tip. Columns are available in two sizes, 1000+ (for use with 1 ml pipettors) and 200+, (for use with 200 µl pipettors) containing 10 and 5 microliters of affinity resin, respectively. The choice in column size provides flexibility in enrichment capacity and throughput.



The design of the columns allows for reproducible and predictable fluid flow through the micro-volume of affinity resin. The images above show a dye solution to illustrate the elution process within the tips. 10 µl of elution buffer is placed in the bottom of a 96 or 384 well plate **(A)**. After loading and washing steps are complete, the elution buffer is passed repeatedly through the bed to maximize recovery from the column. The characteristics of the bed and screen design maintain an intact volume of elution buffer **(B-D)**, allowing for high enrichment factors and reproducible performance.

Rapid Enrichment Process

PhyTip™ columns from PhyNexus can be used in applications ranging from simple low throughput manual pipettors (**A**) to multi-channel electronic pipettors (**B**) and fully automated 96-well robotic workstations (**C**). These unique devices are capable of preparing concentrations of up to 5mg/ml of purified proteins in small final volumes e.g. 10-20 ul, in less than 20 minutes. The column format can process a varying range of initial sample volumes from tens of microliters up to several milliliters.

An example Protein A enrichment method is described below for 200 and 1000 µl sample volumes.

PhyTip™ Rapid Enrichment Protocol

	200+ PhyTip ™	*1000+ PhyTip™*
Capture	*2.:12*	*11:00*
Purify 1	*2:12*	*2:10*
Purify 2	*2:12*	*2:10*
Enrich	*2:24*	*2:20*
Total time (minutes)	*9:00*	*17:30*

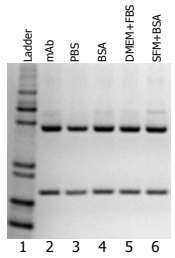
Protein A PhyTip™ Binding Capacity

The PhyTip™ columns were characterized with respect to their maximum binding capacity. A titration of monoclonal murine IgG_{2a} in 500 µl PBS was processed, demonstrating IgG binding capacities exceeding 100 µg. It was determined at low levels of IgG (10 µg) that recoveries on the order of 70% of initial antibody is possible in final volumes of 20 µl (15 µl low pH elution buffer + 5 µl 200 mM phosphate neutralization buffer).

Initial IgG (µg)	Breakthrough IgG (µg)	Bound IgG (µg)	% Bound
10	<2	>8	>80
50	4	46	92
100	16.5	83.5	84
150	47.5	102.5	68
200	71	129	65

High Performance Maintained Across Multiple Affinity Resin Types

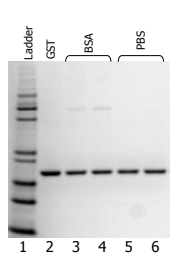
Protein A



Reducing SDS-PAGE gel of a murine mAb IgG_{2a} enriched from varying levels of background proteins. The heavy and light chain bands of the pure IgG shown in lane 2. Lane 3 contains mAb after enrichment from PBS. The remaining lanes show the purified mAb after enrichment from 5 mg/ml BSA (lane 4), DMEM+10% FBS (lane 5), serum-free media with 5 mg/ml BSA.

No background proteins are detected in the enriched samples.

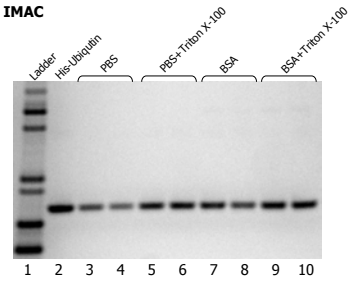
Glutathione



SDS-PAGE gel of GST enriched from varying levels of background proteins using Glutathione PhyTip™ columns. Purified protein was eluted from the column with 20 mM reduced glutathione.

Lane 2: 2 µg pure GST. Lanes 3,4: Duplicate enrichments of a 25 µg/ml sample of GST from PBS with 1 mg/ml BSA. Lanes 5,6: Duplicate enrichments of a 25 µg/ml sample of GST from PBS. Selective enrichment of GST is achieved in the presence of 40X excess of BSA.

IMAC

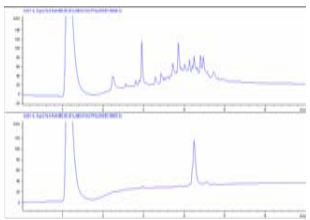


SDS-PAGE gel of His-tagged ubiquitin (His-Ub) enriched from varying levels of background proteins using IMAC PhyTip™ columns. Purified protein was eluted from the column with 250 mM imidazole. 10 µl of each sample was loaded per lane.

Lane 2: 200 µg/ml of the pure His-Ub.
Lanes 3,4: Duplicate enrichments of a 5 µg/ml sample of His-Ub from PBS.
Lanes 5,6: Duplicate enrichments of a 5 µg/ml sample of His-Ub from PBS with 1% TritonX-100.
Lanes 7,8: Duplicate enrichments of a 5 µg/ml sample of His-Ub from PBS with 1 mg/ml BSA.
Lanes 9,10: Duplicate enrichments of a 5 µg/ml sample of His-Ub from PBS with 1 mg/ml BSA and 1% Triton X-100.

No background proteins are detected in the enriched samples. Note that the pre-enrichment concentrations of samples in lanes 3-10 was 40X lower than the concentration of pure His-Ub in lane 2.

Enrichment from Complex Samples



Reverse-phase HPLC trace of GST purification from E. coli whole cell lysate before (top) and after enrichment (bottom) with Glutathione PhyTip™ technology. The data show that the columns achieve high levels of selectivity and enrichment from crude samples.

Summary

PhyTip™ columns are highly effective, simple tools that can be used to purify and enrich antibody and protein preparations at a micro-volume scale with unprecedented speed. With the exponential improvements in enrichment capability and simple adoption to variable formats, i.e., adaptable to various fluid handling formats, application of this technology allows for truly high-throughput capabilities for antibody and protein purification for downstream functional and analytical studies.