



PhyTip[®] 5K Gel Filtration column for automated high throughput desalting & buffer exchange of functional proteins

High Performance:

- Remove >95% of salts with >80% yield of protein
- Retain protein functionality
- <10% CV in volume and concentration
- Ready for downstream assay

Automated desalting/buffer exchange of functional proteins:

- Fully automated process with MEA Personal Purification System
- 12 samples desalted/buffer exchanged in ~30 minutes.

High throughput format:

- Available in 96 sample format for high throughput processing



Introduction

For biopharmaceutical companies whose focus is on antibody and recombinant protein molecules, there is an increasing desire to perform more screens of potential leads where the data generated are more physiologically relevant to the issues of potency, toxicity, and other factors. These assay formats all require that the antibodies and recombinant proteins are well purified, enriched and functional. By utilizing high-performance micro scale functional protein separations, it is now possible to obtain more physiologically relevant data at the high-content screening step and thus make the decision-making power available at the earliest stages in the discovery process. PhyNexus PhyTip column technology has been developed for high-throughput preparation of antibodies and recombinant proteins to facilitate the process of

preparing large numbers of potential leads that are ready for functional assays without the need for scale-up.

In many cases differing elution conditions are required for various downstream functional assays. Furthermore, the final purification product may require an additional preparation step to either reduce toxic buffer salts or to completely exchange buffer conditions. This latter step can now be achieved through fully automated desalting and buffer exchange with PhyTip 5K gel filtration columns used in conjunction with the MEA Personal Purification System. These unique columns allow for high recovery of the functional antibody of interest while removing greater than 95% of salts.

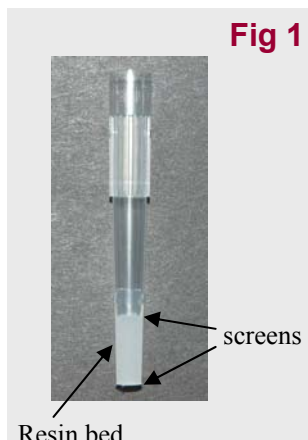


Fig 1 PhyTip 5K Gel Filtration Columns

The design of the patent pending 5K gel filtration columns (Fig 1) is based upon a unique manufacturing process where pipette tips are filled with gel filtration resin. Thin screens are placed above and below the resin bed that retain the gel filtration media within the column structure, while allowing the passage of liquids. The top frit is a larger liquid *interface barrier* that helps facilitate the transfer of liquid to the top of the column. PhyTip 5K gel filtration columns retain small molecules while molecules over 5 KDa will pass through the gel filtration media.

Desalting or Buffer Exchange Process

Using the MEA Personal Purification System, a row of either 200 μ L or

resin bed PhyTip 5K gel filtration columns are conditioned in 1mL of buffer. After the columns are conditioned, samples are added and processed followed by differing volumes of chaser buffer addition and processing. The samples can be collected in fractions to determine the optimal sample recovery and salt removal conditions. Using the MEA Personal Purification System, each row of 12 samples requires 30 minutes to process while the entire plate of 96 samples can be processed in 2.5 hours.

Results

The process of desalting or buffer exchange using the PhyTip 5K desalting columns and the MEA Personal Purification System have been carefully optimized to ensure both maximum recovery of the protein of interest and removal of salt.

Consistent Volume Recovery

Most pharmaceutical companies streamline the processes of antibody/protein production, purification, and desalting/buffer exchange for cell-based assays. In order to efficiently process the samples from production to cell-based assays, high throughput solutions are desirable. However, in practice, achieving a true high throughput solution requires a level of precision not easily achieved. High throughput sample preparation is not advantageous if subsequent quantification is required prior to the next assay. The solution requires consistent mass and concentration recovery.

To determine the consistency of volume recovery from 200 μ L resin bed PhyTip gel filtration columns, 80 μ L or 130 μ L of buffer was loaded on top of 11 or 12 conditioned columns. Flow through was collected and the volume was measured. In three replicate experiments, recovered volumes varied by less than 10% CV (Fig 2A & 2B).

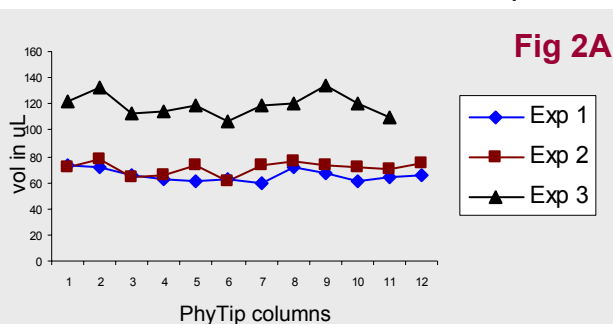


Fig 2A

	Fig 2B		
Chaser Vol	E1: (80uL)	E2: (80uL)	E3: (130uL)
Column1	73.4	70.9	122.6
Column2	71.4	77.4	132.6
Column3	65.9	64.4	112.6
Column4	61.8	65.2	115.0
Column5	61.6	73.8	118.8
Column6	61.9	61.6	106.5
Column7	60.0	73.6	119.4
Column8	71.4	76.7	120.6
Column9	67.1	72.6	133.4
Column10	61.6	71.6	121.0
Column11	64.7	70.7	110.0
Column12	65.4	74.9	
Average	65.5	71.1	119.3
SD	4.5	5.0	8.4
CV	6.9	7.0	7.0

Separation by PhyTip gel filtration column

Sample (far left microfuge tube) containing brown myoglobin protein (16.7kDa) and yellow DNP-glutamate salt (313Da) was loaded onto a 600uL PhyTip gel filtration column. The same PhyTip column at different steps of desalting is pictured along with the collected flow through. From left: 1) column conditioned by PBS buffer prior to sample loading, 2) column after 200µL sample has entered the resin bed, 3-6) column after 100µL PBS buffer is applied, 7) column after a final chase of 400uL PBS buffer. This example illustrates a typical separation allowing the user to discard sample flow through (2) to maintain concentrated purifications. Additionally, salt is retained until fraction (6), while most of the protein is released by fraction (4).

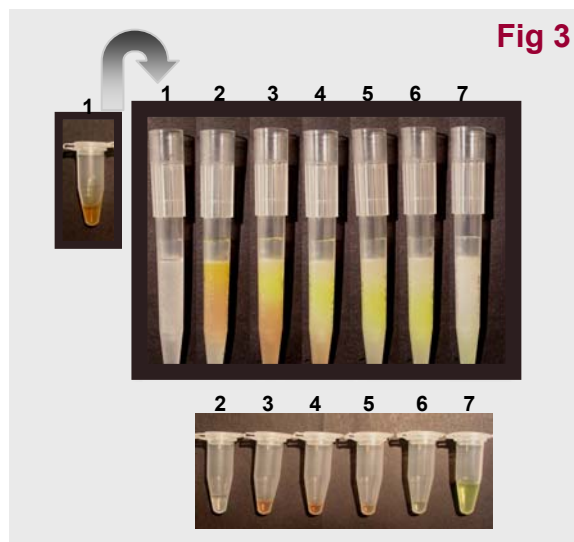


Fig 3

HPLC chromatogram (at right) of His-Tagged ubiquitin at 0.05mg/mL concentration spiked with 250mM imidazole. 300µL samples (red) were applied to 600µL columns. The resulting desalted sample was collected and adjusted to 300µL with water (blue) to compare with the starting sample. Water was injected as a blank (green). The Post-desalting sample shows both recovery of protein and complete removal of imidazole.

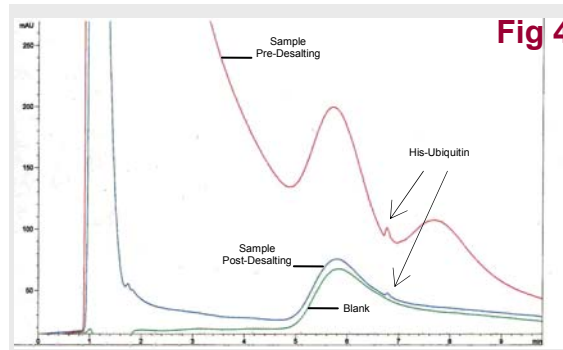


Fig 4

Myoglobin recovery and salt removal

After columns are conditioned, samples are first added and processed followed by different volumes of chaser buffer addition and processing. The samples can be collected in fractions to determine the maximum sample recovery. In this experiment, we used myoglobin and DNP-glutamate (20µL sample volume) and separated myoglobin from DNP-glutamate using 80µL chaser volume (Table 1) and 200µL resin bed gel filtration columns. As shown in the table below, we were able to recover ~80% of myoglobin and remove ~99.8% of DNP-glutamate. Using the MEA Personal Purification System each row of 12 samples can be separated in about 30 minutes. A full plate of 96 samples takes about 2.5 hours to process.

Table 1:

	A364	A409	Volume	pmol Myo	mol Salt	% prot rec	%salt rem
Myoglobin input		1.165	20.0	47843.9			
Myoglobin sample1		0.205	90.5	38095.5		79.6	
Myoglobin sample2		0.200	94.8	38932.2		81.4	
DNP-glutamate input	2.440		20.0		70469.3		
DNP-glutamate sample1	0.003		88.7		96.1		99.9
DNP-glutamate sample2	0.006		89.3		193.4		99.7

IgG separation and recovery

IgG was separated from free dye using a 200 μ L resin bed gel filtration column. In this experiment, PhyTip gel filtration columns were first conditioned with 800 μ L of PBS. After conditioning, 40 μ L of [2mg/mL] sample was added and processed followed by 130 μ L of chaser (PBS) buffer to the PhyTip gel filtration column to collect and separate IgG from unlabeled dyes.

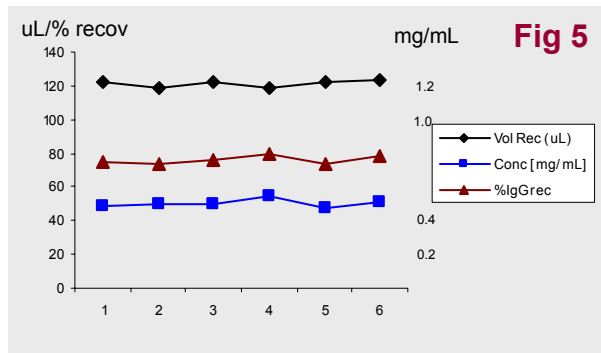


Figure 5 and Table 2 demonstrate that we were able to recover an average of 76% of IgG (Table 2, Fig. 5 red) with an average of 0.5mg/mL concentration (Table 2, Fig. 5 blue) in an average final volume of 121 μ L (table 2, fig 5 black). As shown in Table 2, the percent IgG recovery, concentration, and final recovered volume had very small CV values. These SD and CV values show the reliability and consistency of the PhyTip gel filtration columns.

Table 2

Column #	Final Volume [μ L]	Concentration [mg/mL]	Mass Recovered (mg)	% Protein Recovered
1	122	0.49	0.060	75
2	119	0.50	0.060	74
3	122	0.50	0.061	76
4	119	0.54	0.064	80
5	122	0.48	0.059	73
6	123	0.51	0.063	78
Ave	121	0.50	0.061	76
SD	2	0.02	0.002	3
CV	1.4	4.1	3.5	4

Conclusion

PhyTip 5K gel filtration columns and the MEA Personal Purification System represent a high throughput solution for rapid desalting and buffer exchange. PhyTip 5K gel filtration columns can process 12 samples in 30 minutes compared to the laborious and time consuming dialysis procedure. Using PhyTip 5K gel filtration columns, researchers can save hours while obtaining a high level of performance. Unlike spin columns, PhyTip columns simulate traditional chromatography and can perform fractionation of protein samples. This allows researchers to optimize maximum protein recovery and salt removal in an automated, high throughput manner.

PhyTip 5K gel filtration columns can be used in combination with PhyTip purification columns on the MEA Personal Purification System. This allows researchers to combine purification with desalting and buffer exchange. For example, 6-His-tagged proteins can be purified by PhyNexus IMAC PhyTip columns and eluted using high concentrations of imidazole, which can be removed by PhyTip gel filtration columns. These processes allow researchers to perform multiple sample processing steps in a high throughput, walk-away scenario.