PhyTip® ProPlus Affinity Columns... the antibody purification system that is the best of Protein A and Protein G

- Capture, purify and enrich in as little as 15 minutes to obtain high concentrations of fully functional protein
- Process small sample volumes in a reproducible, high throughput, automated format
- Elution volumes as low as 10 µL, producing enrichment factors as high as 50 fold, with concentrations of purified protein of up to 10 mg/mL
- At least 10% higher capacity than Protein A resins
- Broad range of selectivity, recognizing various isotypes and species, similar to Protein G
- 3-5 fold greater recovery than Protein G

Introduction

The PhyNexus PhyTip® columns are innovative purification tools that radically simplify the capture, purification and enrichment of proteins from a variety of sources. Key to the success of these purification tools is the design of the mechanism to retain the affinity resin bed, with minimum dead volume and maximum capture potential. One group of existing PhyNexus products include PhyTip columns containing Protein A or Protein G affinity resin. These specifically bind antibody (IgG) from different sources under certain optimal conditions, thus allowing nucleic acids and other contaminants to be removed. Following a rapid wash step, purified antibodies are easily eluted with commonly used low pH buffer. This technique allows for exceptionally high yields of IgG, depending on various conditions and provides for highly selective purification. PhyTip columns have extremely high binding capacity that can bind up to a few mgs of IgG, and can efficiently recover as little as 200 ng of IgG.

The ProPlus resin ligand is a Protein A derived ligand that demonstrates attributes of both Protein A and Protein G. Protein G demonstrates superior selectivity over Protein A for certain isotypes and species of IgGs, such as mouse and rat IgGs. However, Protein G has lower capacity and therefore is not as desirable when higher recovery is required. The new PhyTip ProPlus columns demonstrate similar selectivity to Protein G and higher capacity than Protein A allowing for the best of both worlds. PhyTip ProPlus columns are ideal for antibody screening from hybridoma supernatants or for high throughput extraction and purification of expressed antibodies.

IgG binds ProPlus with high affinity and specificity. The strength and selectivity of this interaction enables ProPlus to effectively purify IgGs from complex protein mixtures. To examine the performance of PhyTip columns with ProPlus resin, the percent recovery of purified IgGs using 1000+ ProPlus PhyTip columns was measured.

Materials and Methods

Sample processing

All 1 ml samples were processed with 20, 40 or 80 µl columns on the automated MEA 1000 platform using the following protocols:

1. Capture: Capture the specific antibody by passing the 1 ml sample over the resin bed with 4 in/out cycles @ 0.5 ml/min.

2. Purify: Remove unbound proteins by washing the bound protein/affinity resin using 1 in/out cycle of 1 mL PBS @ 0.5 ml/min (Wash Buffer I) followed by 1 in/out cycle with 1 mL of saline solution @ 0.5 ml/min (Wash Buffer II).

3. Enrich: Elute the specific antibody with 4 in/out cycles @ 0.5 ml/min with 60 µl (for 20 µl columns), 120 µl (for 40 µl columns) or 240 µl (for 80 µl columns) of pH 2.5 elution buffer.

Once eluted, 15 µL (for 20 µl columns), 30 µl (for 40 µl columns) or 60 µL (for 80 µl columns) of pH 9.0 neutralization buffer was added.

Quantitation procedure

1. 80 µl of eluted antibody sample was injected into a non-porous polystyrene divinylbenzene reverse phase column using an HP 1050 HPLC system. A gradient of 15% to 85% between solvent A (0.1% TFA in water) and solvent B (0.075% TFA in ACN) was used for 10 minutes. Detection: UV at 214 nm.

2. Antibody peaks eluted around 7 min. The area under this peak was integrated and corresponding peak area was recorded at 214 nm.

3. Antibody standard under identical reaction conditions was loaded into the column and used as an input or standard for recovery calculation.

Results

Selectivity Experiment

The recovery of different isotypes and species of IgG by PhyTip ProPlus columns was compared to the recovery by Protein A and Protein G columns, as shown in Figure 1 and Table 1. Protein G is superior to Protein A for selectivity of certain isotypes of IgGs including mouse isotypes. We have shown that ProPlus demonstrates a similar selectivity to Protein G for the $mIgG_1$, $mIgG_{2a}$ and hIgG isotypes used here. In addition the capacity and recovery of functional antibody is superior to both Protein A and Protein G columns.

Samples

1ml PBS, 0.05% Tween containing 5 µg of either:

1. mouse IgG_1
2. mouse IgG_{2a}
3. human IgG

were added to 20 µl columns (for Figure 1 and Table 1), and 20 µl or 80 µl columns for Figure 2 and Table 2.

Reproducibility

To demonstrate reproducibility, each antibody was purified in three experiments (n=3) of each resin type. The %CV was below 18% for all, and was an average of 7.5%.

Figure 1. ProPlus, ProA and ProG Selectivity

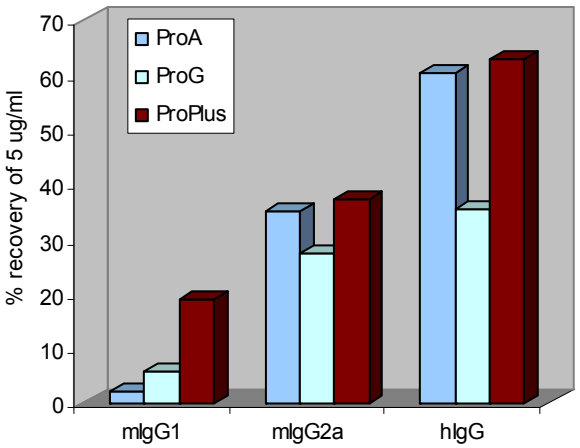


Table 1	$mIgG_1$			$mIgG_{2a}$			hIgG		
	ProA	ProG	ProPlus	ProA	ProG	ProPlus	ProA	ProG	ProPlus
%recovery	2	5.6	18.9	35	27.3	37.1	60.4	35.4	62.9
SD	0.36	0.59	1.09	1.73	0.65	2.21	3.81	1.07	6.99
%SD	17.6%	10.6%	5.8%	5%	2.4%	6%	6%	3%	11.1%

Capacity as a function of Resin bed Size

A larger resin bed size provides higher capacity of antibody binding and therefore results in greater recovery of antibody. Dilute samples such as those analyzed here may benefit from a larger resin bed size. However, a larger elution volume is required as well, causing the elution fraction to be larger and more dilute than that obtained with a smaller resin bed volume column. The data in Figure 2 and Table 2 demonstrates the differerence in recovery between purifications using 20 µl columns and 80 µl columns.

Figure 2. mIgG$_1$ recovery, 20ul & 80ul columns

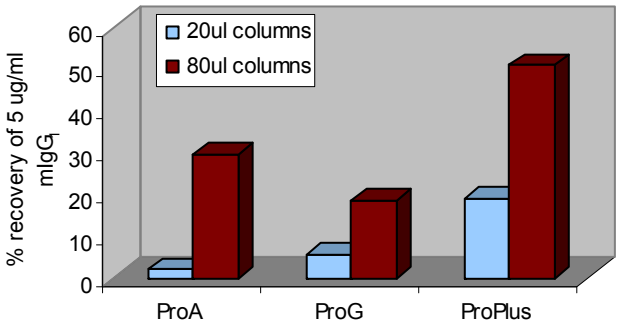
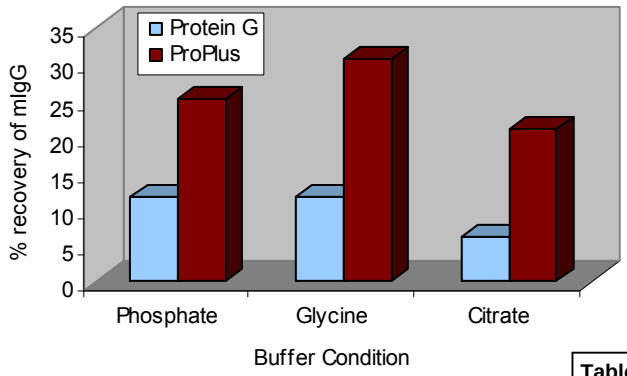


Figure 3. ProPlus and Protein G mIgG recovery



Mouse Hybridoma Experiment

This experiment shown in Figure 3 and Table 3 demonstrates the application of ProPlus columns for the screening of mouse hybridoma samples. The 3-5 fold increase in recovery using ProPlus columns compared to Protein G dramatically demonstrates the utility of these new columns. Three different pH 2.5 elution buffer conditions were examined, Phosphate, Glycine and Citrate Buffers.

Samples

1. 0.05mg/mL mIgG$_1$, low bovine IgG media
2. 0.05mg/mL mIgG$_1$, 10% FBS IgG media
3. mIgG$_1$ clone expressor, low bovine IgG media
4. mIgG$_1$ clone expressor, 10% FBS IgG media
5. (-) control, low bovine IgG media
6. (-) control, 10% FBS IgG media

Conclusions

1. ProPlus columns are selective for various species and isotypes of IgG, similar to Protein G, and can purify mouse IgGs from hybridomas.

2. ProPlus demonstrates high capacity for bound IgGs.

3. The resin bed volume of PhyTip ProPlus columns can be adjusted to recover the maximum amount of antibody.

Table 2	20ul columns			80ul columns		
	ProA	ProG	ProPlus	ProA	ProG	ProPlus
%recovery	2	5.6	18.9	29.42	18.6	50.1
SD	0.36	0.59	1.09	0.89	0.65	2.21
%SD	18%	10.6%	5.8%	3%	2.4%	6%

Table 3 Mouse hybridoma samples	Phosphate Buffer			Glycine Buffer			Citrate Buffer		
	ProG	ProPlus	ProPlus Factor Increase	ProG	ProPlus	ProPlus Factor Increase	ProG	ProPlus	ProPlus Factor Increase
	Total µg			Total µg			Total µg		
1	12	53	4	7	55	8	4	13	3
2	12	41	3	7	42	6	3	38	13
3	15	26	2	9	21	2	8	11	1
4	27	69	3	20	55	3	10	22	2
5	2	7		2	4				
6	2	5		2	8				
	Average fold greater recovery:		3			4			5