True Parallel Protein Purification with PhyTip® Columns Using the Freedom EVO® and MultiChannel Arm™ 96

- 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.

Introduction

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. Existing PhyNexus products include PhyTip columns affinity media for the purification of antibodies and tagged proteins, as well as conventional chromatography media for ion exchange and size exclusion chromatography, and hydrophobic separations.

Use of PhyTip columns, in conjunction with a Freedom EVO 100, 150 or 200 liquid handling platform equipped with a MultiChannel Arm™ 96 (MCA 96) module, offers a high throughput automated solution for sample preparation and protein purification. For example, 96 hybridoma supernatants can be purified in as little as 15 minutes, thanks to the parallel pipetting capabilities of the MCA 96. The yield, purity and reliability of this procedure make it the ideal platform for sample preparation prior to high throughput applications such as antibody lead screening.

The protein purification process is streamlined for ease of use. PhyTip columns are loaded onto the Freedom EVO platform, and undergo an equilibration step prior to sample addition.

Following sample capture and a rapid wash step, the purified proteins can be eluted with commonly used elution buffers. This technique offers exceptionally high protein yields, depending on the conditions, as well as highly selective purification.

PhyTip columns also have extremely high binding capacities, allowing efficient recovery of antibodies and proteins at concentrations as low as 200 ng/mL. Combining the flexible suite of PhyTip column products with the reliability of the Freedom EVO can significantly increase the productivity of drug development processes.

Materials and Methods

Samples and Reagents – 6xHis-tagged Protein Purification

All methods were developed at PhyNexus, Inc., San Jose, USA.

All 6xHis-tagged protein samples consisted of buffer (0.05% Tween-20 in PBS, 50 µL) spiked with His-tagged ubiquitin (His-Ub, Boston Biochem, U-530) to a final concentration of 0.8 mg/mL. The following buffers were used to process PhyTip columns packed with IMAC resin:

Equilibration Buffer
PBS

Wash Buffer 1
10 mM sodium phosphate, pH 7.4,
500 mM NaCl, 5 mM imidazole

Wash Buffer 2
10 mM sodium phosphate, pH 7.4,
500 mM NaCl, 20 mM imidazole

Enrichment Buffer
10 mM sodium phosphate, pH 7.4,
140 mM NaCl, 500 mM imidazole

Sample processing on PhyTip columns was carried out on the Freedom EVO workstation using a MultiChannel Arm with a 96-channel head and Freedom EVOware® 2.3. The following procedures were followed:

1. Equilibrate: PhyTip columns were washed by passing PBS buffer (0.2 mL) over the resin bed with one aspirate and dispense cycle at a flow rate of 8.3 µL/second, pausing for 10 seconds after each aspirate and dispense step.
2. Capture: His-Ub was captured by passing sample (50 µL) over the resin bed with eight aspirate and dispense cycles at a flow rate of 4.0 µL/second, pausing for 10 seconds after each aspirate and dispense step.
3. Purify: PhyTip columns were initially washed with Wash Buffer 1 (0.2 mL) with one aspirate and dispense cycle at a flow rate of 8.3 µL/second, pausing for 10 seconds after each aspirate and dispense step. This was followed by a second wash with Wash Buffer 2 (0.2 mL), using the same procedure.
4. Enrich: His-Ub was eluted by passing Enrichment Buffer (40 µL) over the resin bed, with eight aspirate and dispense cycles at a flow rate of 4 µL/second, pausing for 10 seconds after each aspirate and dispense step.

Aliquots (2.2 µL) of the final eluate containing His-Ub were analyzed by UV absorbance at 280 nm. A standard curve was generated with known concentrations of His-Ub.

Results

PhyTip® columns are processed via a unique method where samples, wash and elution buffers are aspirated and dispensed back and forth through the resin bed. Manipulation of the flow rate, pauses and the number of cycles allows fine control of the PhyTip columns, and this data is capable of determining scale-up purification conditions.

Reproducibility

To test the reproducibility of the PhyTip column procedure on the Freedom EVO/MCA 96, 24 identical samples were processed. Samples (50 µL) were generated by spiking 40 µg of His-tagged ubiquitin into 0.05% Tween-20 in PBS. PhyTip columns were processed simultaneously using the MCA arm, as detailed. The resulting elution volumes were determined, and absorbance was measured at 280 nm. Under these conditions, over 74% of the protein was recovered and the elution volumes and concentrations showed reproducibility (% CV) of 5 and 10 respectively (Table 1).

	A_{280}	µg/µL	Vol. (µL)	Tot. (µg)	% rec.
1	0.012	0.67	40.1	26.9	67
2	0.012	0.67	38.4	25.8	64
3	0.014	0.81	38.8	31.2	78
4	0.014	0.81	37.4	30.1	75
5	0.016	0.94	37.3	35.0	88
6	0.015	0.87	39.1	34.1	85
7	0.014	0.81	36.5	29.4	73
8	0.014	0.81	36.4	29.3	73
9	0.013	0.74	37.0	27.3	68
10	0.012	0.67	36.6	24.6	61
11	0.013	0.74	37.8	27.9	70
12	0.014	0.81	36.4	29.3	73
13	0.014	0.81	36.8	29.6	74
14	0.013	0.74	35.5	26.2	66
15	0.018	1.07	31.7	34.0	85
16	0.012	0.67	36.3	24.4	61
17	0.014	0.81	36.9	29.7	74
18	0.014	0.81	35.2	28.3	71
19	0.016	0.94	36.0	33.8	85
20	0.015	0.87	36.6	31.9	80
21	0.014	0.81	35.7	28.8	72
22	0.014	0.81	35.3	28.4	71
23	0.017	1.01	33.4	33.6	84
24	0.014	0.81	34.8	28.0	70
Mean		0.81	36.5	29.5	74
SD		0.1	1.8	3.0	8
CV (%)		13	5	10	10

Table 1. PhyTip Tecan 200+ 5mL IMAC reproducibility testing.

Conclusions

1. PhyTip columns perform best using back and forth aspirate and dispense cycles for optimal capture, wash and elution of target proteins.
2. PhyTip columns used in conjunction with the Freedom EVO are capable of achieving reproducible purification results.
3. The Freedom EVO is suitable for processing PhyTip columns, enabling complete automation of sample preparation and protein purification.

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