APPLICATION NOTE

Developed in collaboration with:

YUMAB

flow robotics

Biotage

PROTEIN PURIFICATION USING PHYTIP® COLUMNS ON THE FLOWBOT® ONE

1. INTRODUCTION

Automating small-scale purification can enable more research, shorten workflows, free up time for other tasks, and standardize results. One effective way of automating protein purification is by utilizing PhyTip® columns, manufactured and sold by Biotage®, on a robotics platform.

In this application note, we will demonstrate our development of an automated protein purification solution using the flowbot® ONE* and PhyTip® columns. Specifically, we will present purification of human IgG samples using 1000 µL PhyTip® ProPlus with a 40 µL resin bed.

These were used to successfully purify human IgG with satisfactory yields up to 82%, depending on the sample characteristics. We developed two template programs for 4 and 24 samples, respectively.

In such programs, we carefully tuned key features of the flowbot ONE to achieve optimal flow rates and the number of aspiration-dispense cycles of samples and buffers through the tips. Any user who would like to run the same protocol would simply need to adapt the template program to the number of their samples.

Fortunately, accomplishing this is straightforward on the flowbot ONE as the user-friendly software and built-in liquid-class editor facilitate easy control of these parameters.

Indeed, the flowbot ONE offers the flexibility to process 1, 4 or 8 samples simultaneously, depending on the chosen pipette module configuration.

This work was conducted in collaboration with YUMAB, a Contract Research Organization (CRO) which specializes in human antibody discovery and engineering technologies.

2. MATERIALS AND METHODS

PhyTip® columns dual chromatography is based on three steps – capture, wash and elute, with an equilibration step as column preparation.

In each step, the liquid is cycled through the PhyTip® columns where it interacts with the affinity resin. Automating this process entails configuring the number of aspiration-dispense cycles for each step and controlling the pipetting flow rates.

The flowbot ONE software allows for the addition of the necessary number of cycles using the mixing function. Using the integrated Liquid Class Editor enables precise adjustments of the flow rates for the liquid classes used in each step.

In this experiment, a protocol on the flowbot ONE was set up to purify 4 human IgG samples suspended in 3 mL culture medium using the 1000 µL ProPlus PhyTip® with 40 µL resin. The execution time for the protocol time was 48 minutes and 52 seconds.

The samples were spiked with 500, 200, 60 and 32 mg/L polyclonal human IgG. The experiment was repeated using samples suspended in PBS as a control.

A program was easily created for the flowbot ONE to follow pipetting flow rates and cycles as seen in Table 1.

The settings were decided based on recommendation by Biotage® as well as through an optimization process taking both run time and result quality into account.

With the intention to maximize total yield, a sample volume of 3 mL was used as input in comparison to many other approaches, where only 1 ml sample is processed.

flow robotics

Table 1. Overview of settings used in the experiment on the flowbot ONE. For equilibration, a standard PBS buffer was used. For the washing, 0.2 M NaCl was used. As elution buffer 50mM phosphoric acid (pH 2.8-2.9) was used. Lastly, a neutralization buffer of 50 nM Na_2HPO_4 + 0.75 M NaCl at pH 11 was used, resulting in neutral pH buffer with PBS-like conditions when mixed with the elution buffer.

Step	Mix volume (µl)	Cycles	Flow rate (µl/s)	Aspiration Delay (s)	Dispense Delay (s)
Equilibration	950	1	16	20	20
Capture	950 x 3	3 x 3	16	20	20
Wash	950	1	16	20	20
Elution	240	4	16	20	20
Neutralization	60	1	16	-	-

Additional capturing steps were included to ensure thorough capturing. The samples were therefore captured in three steps allowing us to process a larger sample volume for a higher total yield.

A volume of 950 µL was transferred to a "processing plate" and cycled three times. This process was repeated to achieve a total of 3x950 µL cycled through the tip before continuing to the wash step.

The labware used in these experiments were based on YUMAB's preferences and easily added to the flowbot ONE's software by using the built-in Component Editor.

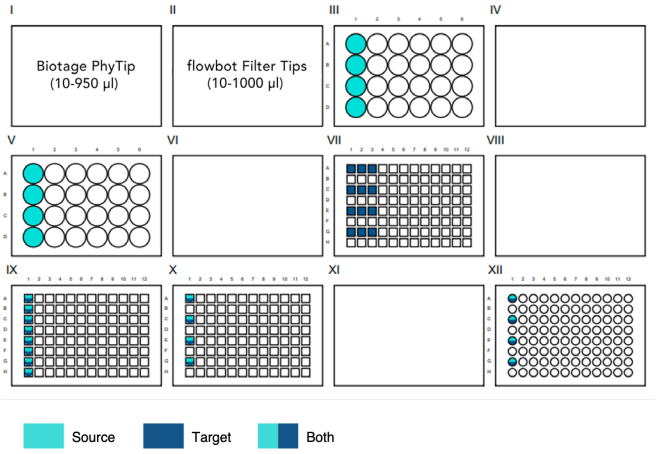
Programs can easily be tailored to meet specific labware requirements by using the Component Editor in the software.

Figure 1 illustrates the layout of the program on the flowbot ONE deck. After the final neutralization step on the flowbot ONE, the purified antibodies were quantified by an absorbance reading of A280 nm, and the purification efficiency was calculated. The purity and integrity of the antibodies were checked by reducing SDS-PAGE analysis.

An additional template program has been designed to allow for the purification of twenty-four samples (3 hours and 45 minutes). The setup on the flowbot ONE for 24 samples is identical to the one shown in Figure 1.

Two additional 24-well sample plates and two 96-well processing plates can be placed on deck and processed by the robot at the same run. This would enable a convenient overnight purification process of 72 samples.

Figure 1: flowbot® ONE deck layout. Note that positions IV, VI, VIII and XI are empty/free deck space.



Deck details

I: Box with PhyTip® ProPlus

II: Box with flow Robotics tips

III: 24-well plate with neutralization buffer (1-5 ml each)

V: 24-well plate with samples for purification (3 ml each)

VII: Empty 96-well "processing" plate for storage of the processed samples

IX: 96-well plate with equilibration buffer (1 ml each)

X: 96-well plate with washing buffer (1 ml each)

XII: 96-well plate with elution buffer (240 µl each)

The flowbot ONE could further be fitted with an 8-channel pipette module to accommodate for larger sample volumes, as needed. Further customization for other sample types and variants of PhyTip® columns can be done, e.g. different number of capture cycles, elution volumes and flow rates. All of this is easily customized in the software with a few clicks.

3. RESULTS

The recovered IgG is illustrated in Figure 2 + 3 and Table 2. Depending on the initial IgG concentration in the sample and the available total protein amount, a purification yield of up to 82% was achieved (Figure 2).

The two alternative setups - presenting antibody in Medium spiked buffer vs PBS buffer spiking - did not result in significantly altered protein capture and purificaion efficiency. If at all - the medium spiking resulting in slightly higher protein yields.

The highest total yield was achieved from the approximately 1.5 mg sample which allowed for a recovery of 0.67 mg IgG in total, as shown in Figure 3 and Table 2.

Additional analysis by SDS-PAGE under reducing conditions revealed an intact heavy chain (HC) and light chain (LC) of the antibody with the expected size and high purity after purification from the culture medium (Figure 4).

Figure 2: Bar chart of the % recovered protein for each of the four samples suspended in culture medium and PBS

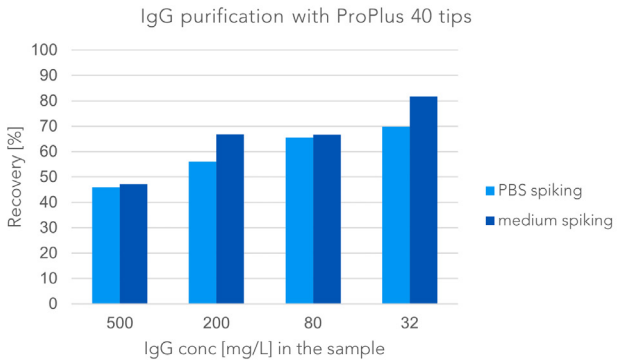


Figure 3: Bar chart of the amount of protein in the initial samples (yellow bars) versus recovered amount of protein in the eluted purified samples (red bars) for samples suspended in culture medium, and recovered amount of protein suspended in PBS (gray bars)

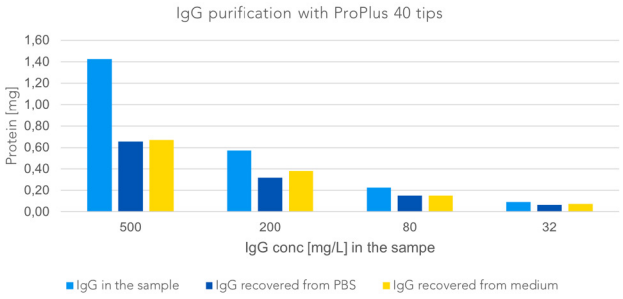


Figure 4: SDS-PAGE reveals an intact heavy chain at 50kDa and a light chain at 25kDa

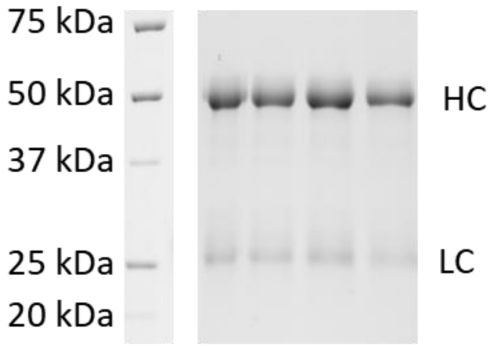


Table 2. IgG input and recovery for all samples as well as %CV between the two independent variables.

PBS spiked sample

Sample	Initial IgG conc [mg/L]	IgG processed [mg]	IgG recovered [mg]	Recovery [%]	End conc in PBS [mg/ml]	Concentration increase	Mean conc. increase (for both samples)
1	500	1.43	**0.66**	**46**	**2.34**	**4.7x**	4.7x
2	200	0.57	**0.32**	**56**	**1.14**	**5.7x**	6.3x
3	80	0.23	**0.15**	**66**	**0.53**	**6.7x**	6.7x
4	32	0.09	**0.06**	**70**	**0.23**	**7.1x**	7.7x

Medium spiked sample

Sample	Initial IgG conc [mg/L]	IgG processed [mg]	IgG recovered [mg]	Recovery [%]	End conc in Medium [mg/ml]	Concentration increase	Mean conc. increase (for both samples)
1	500	1.43	**0.67**	**47**	**2.40**	**4.8x**	4.7x
2	200	0.57	**0.38**	**67**	**1.36**	**6.8x**	6.3x
3	80	0.23	**0.15**	**67**	**0.54**	**6.8x**	6.7x
4	32	0.09	**0.07**	**82**	**0.27**	**8.3x**	7.7x

4. CONCLUSION

With the execution of these experiments, we have shown how the flowbot ONE can perform automated protein purification using Biotage® PhyTip® columns for dual flow chromatography, and how it is simplified with the built-in Component Editor and Liquid Class Editor.

In this specific set-up, the 1000 µL ProPlus tips with 40 µL resin were used to successfully purify human IgG with satisfactory yields up to 82%, depending on the sample characteristics. For samples with a high starting amount of IgG, we naturally see a lower % yield as we are nearing the maximum binding capacity of the resin.

With the initial focus on achieving a high maximum yield, the % yield becomes more redundant since a high total yield of eluted protein (0.68 mg IgG) was achieved as desired.

One way of improving the % yield is to increase the number of capture cycles. However, this will increase run time as well. In this setup, YUMAB needed to process 3 mL to maximize the total protein yield. An alternative could be processing only 1 mL, thereby eliminating the need for a processing plate and freeing up space for a higher sample throughput if less protein is sufficient.

Replicate measurement revealed highly similar recoveries with a CV (coefficient of variation) between 1% and 12% observed for the different samples, confirming good repeatability when using PhyTip® columns on the flowbot ONE.

Analysis by SDS-PAGE indicates that high purity and integrity can be expected using this purification method.

With this study we have created a template program for working with PhyTip® columns which results in high quality protein purification. The program can be made commercially available in the flowbot ONE software, upon request.

The program can be adjusted to accommodate specific needs such as other requirements for labware, number of samples processed, sample volume processed as well as desired changes of flow rates and number of cycles, as well as the different variants of PhyTip® columns.

Optimization using these simple parameters allows to customize the automation for required process yields and sample concentration within the time constraints of a given workflow. All of this can be done using the intuitive software of the flowbot ONE.

For more information about YUMAB please visit www.YUMAB.com

For more information about the flowbot ONE please visit www.flow-robotics.com

For more information about PhyTip® columns, please visit www.biotage.com