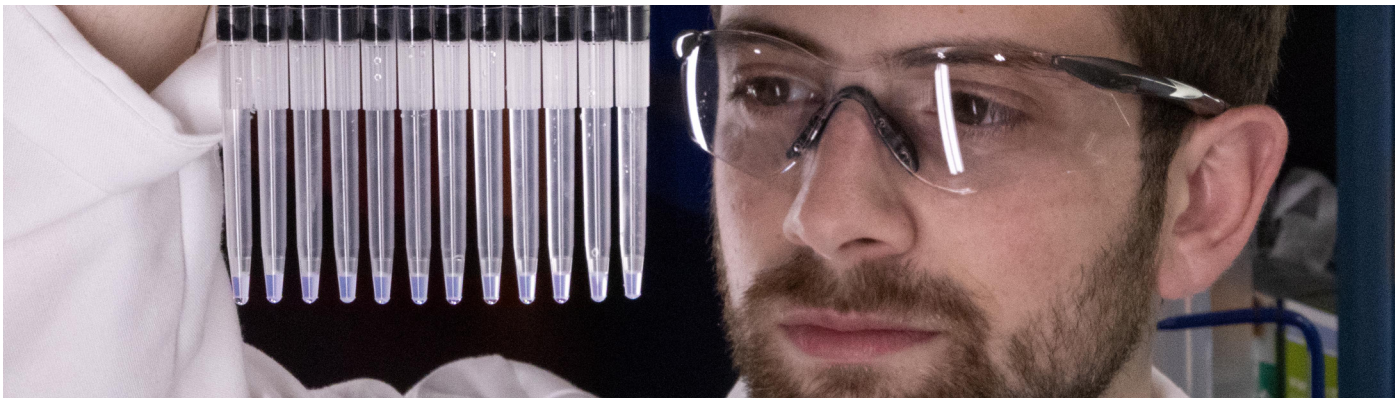
Accelerating High Throughput Protein Purification Using PhyTip® Columns

A White Paper from Biotage

Author: *David Westman*



Introduction

Drug discovery and development is a multifaceted process which involves a long and exhaustive journey through basic research, discovery of the medicine, preclinical development tests, increasingly complicated clinical trials, and finally the regulatory approval. The initial drug discovery and development process includes target validation, high throughput screening of drug candidates, developing assays for therapeutic efficacy, and lead optimization. One of the key aspects in this phase is to become more efficient in finding the hits and shorten the time to identified drugs for process development and scaling up.

For biologics and advanced therapeutics, the purification steps for characterization and drug interaction studies can be a bottleneck. The process for adequate preparation often requires that sufficient quantities of material be scaled up and processed in a time-consuming manner using expensive chromatography equipment. Commonly used purification techniques are magnetic beads and filter plates. However, these technologies suffer from drawbacks such as expensive specific instrumentation for magnetic beads and complex automation protocols for the filter plates. Recent advancements in miniaturized high-throughput tools for purification, enrichment and desalting of proteins eliminate bottlenecks associated with traditional protein purification techniques.

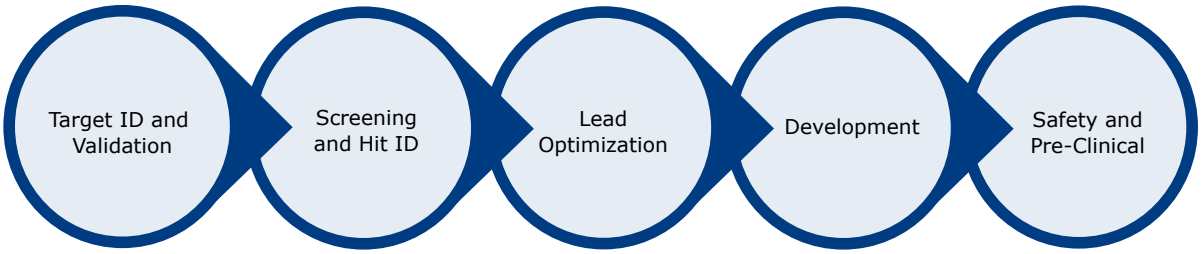


Figure 1. The drug discovery and development process.

Biotage®

Dual Flow Chromatography

Dual flow chromatography (DFC) is the unique process of performing chromatography separations in a column bed loosely packed at the end of a pipette tip, retained by a minimally absorbing, proprietary mesh – a PhyTip® column. Dual flow chromatography works by having a flow through the PhyTip® column and pumping the sample back and forth across the packed resin bed inside the tip. As the sample mixture is cycled back and forth more than once with a controlled flow across the resin bed, the biomolecules in the sample is given enough time to interact with the available binding sites despite the slow transfer kinetics associated with large biomolecules. As a result, all the protein, viral vector or plasmid can be captured during the capture step, leading to very high recoveries at the end of the purification process. And if this is not the case, this is easily adjusted by simply applying more DFC cycles to increase the contact time between the sample and the binding sites on the resin. The same applies to the wash step and elution step (Figure 2) – the number of cycles is an easy way of fine-tuning the purification process for a specific application. And when each step of the protein purification protocol is completed this way and driven to equilibrium, the purification will perform the same every time. That means the results are highly reproducible with the same sample purity and yield every time.

The unique column design with a packed resin bed binds the entire sample to a small resin bed volume. Basic chromatographic principals control the separations, and the bi-directional flow drives interactions to high loading equilibrium regardless of kinetic rate constants. The purified sample can be eluted in small volumes, resulting in a highly concentrated sample. Dual flow chromatography is a gentle purification process producing high levels of biologically active protein.

Automated High Throughput Purification

Biotage' PhyTip® column technology has been developed for small scale high-throughput preparation of biomolecules such as antibodies, recombinant proteins, plasmids and viral vectors in order to facilitate the process of preparing hundreds to thousands of potential leads that are ready for cell-based assays without the need for scale-up. These unique columns are designed to operate on all major liquid handling robot platforms (Hamilton, Tecan, Beckman and more) either on 96-at-a-time platforms or on 8-12 channel robotic platforms, see Figure 3 below. The PhyTip® columns are ready to use directly out of the box. The run time for a full purification protocol of 96 samples can be as short as 15 minutes depending on sample volume. Methods are easy to develop, and validated protocols are available for many of these platforms.

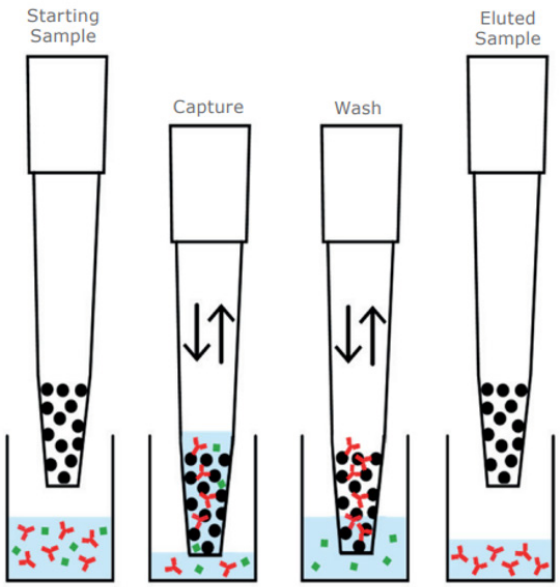


Figure 2. Principles of dual flow chromatography.

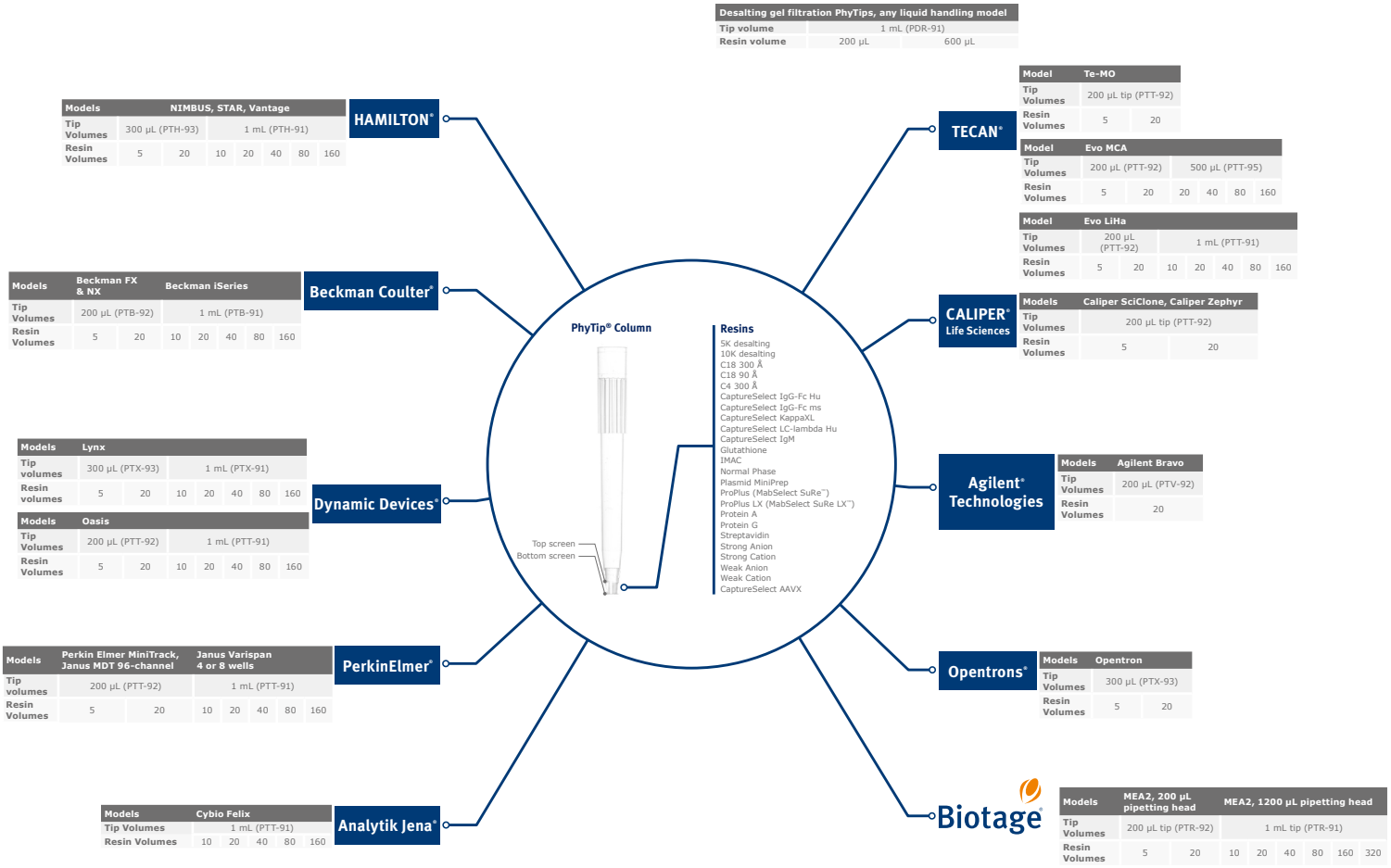


Figure 3. PhyTip® columns are designed to operate on all major liquid handling robots.

Enabling High-Throughput Screening

When automating library screens, researchers have several goals they need to accomplish. They have to express multiple constructs and in multiple expression systems. Also, when optimizing the protein solubility and stability, they need to test many different conditions. In traditional small scale high throughput purification technologies researchers often face challenges with reproducibility, purity and recovery. Here we show examples where PhyTip® columns overcome some of these challenges.

Figure 4 displays some data from a study performed at Roche. This group consistently clones and expresses varying constructs as part of an X-ray crystallography screen and must test different expression systems to identify forms with the highest expression levels, stability, and potency. In this study they were looking at the incorporation of PhyTip® columns in their process. The SDS-PAGE gels demonstrate a level of purity and consistency of the purified constructs across their entire screen.

A. 16 Constructs expressed in E. coli cultures.

B. 9 Constructs expressed in Baculoviral cultures.

C. 3 Constructs expressed in Mammalian cultures.

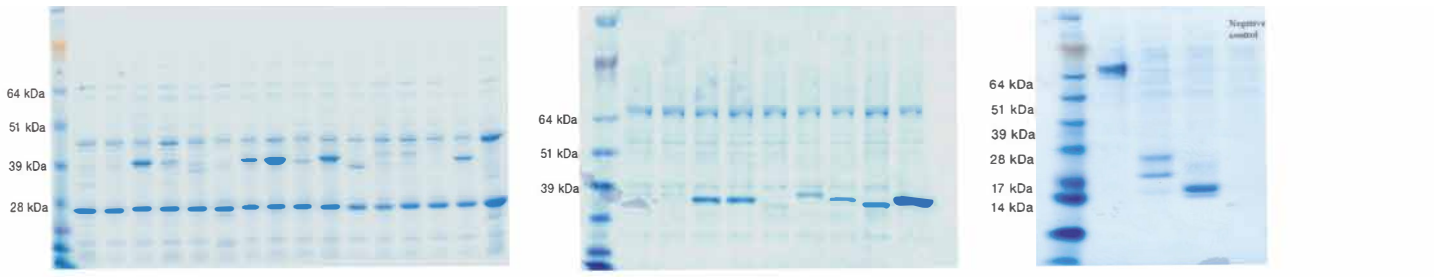


Figure 4. SDS-PAGE gels on protein purity from different constructs expressed in different expression systems.

A.

pH HEPES/ NaOH

mM HEPES/ NaOH

%v/v Glycerol

mM NaCl

pH Tris-HCl/ NaOH

mM Tris-HCl/ NaOH

mM L-Arginine

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
pH HEPES/ NaOH		7.00	7.00	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00
mM HEPES/ NaOH		20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
%v/v Glycerol		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
mM NaCl		150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
pH Tris-HCl/ NaOH	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
mM Tris-HCl/ NaOH	7.00	7.00	7.00	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00
mM L-Arginine	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
	7.00	7.00	7.00	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00
	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
	7.00	7.00	7.00	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00
	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00	8.50	8.50	8.50
	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00	8.50	8.50	8.50
	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00	8.50	8.50	8.50
	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
	7.50	7.50	7.50	7.80	7.80	7.80	8.00	8.00	8.00	8.50	8.50	8.50
	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0	150.0	325.0	500.0
	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0

Figure 5a. Buffer composition of samples for testing protein stability and binding efficiency.

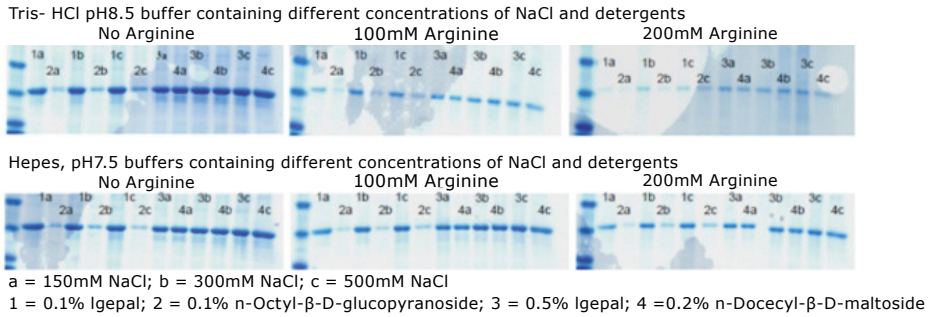


Figure 5b. Impact of NaCl concentrations and detergents.

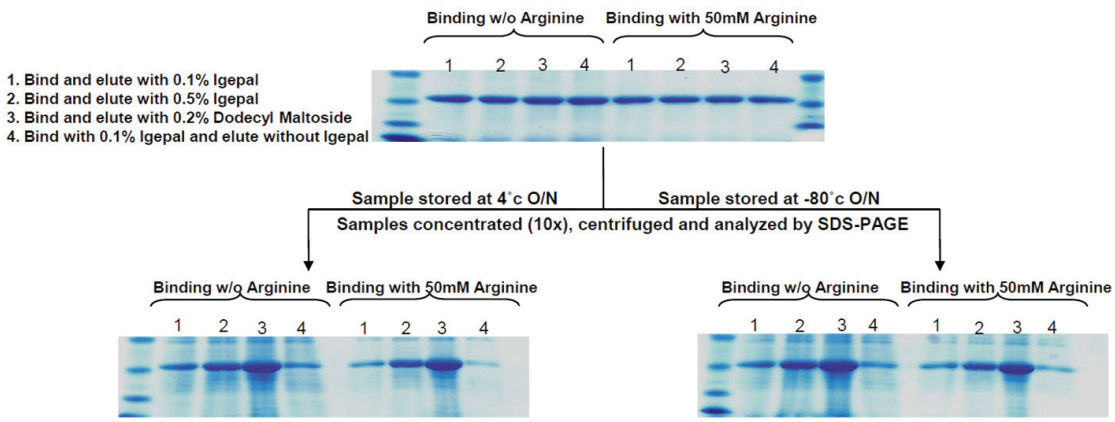


Figure 5c. Impact of arginine for binding efficiency.

In Figure 5, with conjunction with a Hummingbird robot and PhyTip® columns, the same group tested a matrix of buffers for binding efficiency as well as protein stability all in one experiment. Samples from two of the buffers from the panel were tested for stability overnight and analyzed by SDS-PAGE. It was determined the protein most efficiently bound and was most stable in Tris-HCl, pH 8.5 with 0.2% dodecyl maltoside and no arginine.

As a conclusion this application example showed how PhyTip® columns can be used to enable high throughput purification to evaluate binding and stability characteristics with high levels of consistency and purity.

Shortening Immunoprecipitation Workflows

Immunoprecipitation (IP) is a widely used technique to isolate specific types of proteins or a part of a protein containing a specific biomarker from cell or tissue lysates. It is essential for studying post-translational modifications (PTMs) as well as studying drug-target interactions. A specific antibody is immobilized on a bead matrix and used to bind its antigen. Traditional immunoprecipitation is performed in a microcentrifuge tube and each step is carried out in a batch-binding manner with the use of a centrifuge.

The batch-binding technique is difficult to translate on a robotic system which is why PhyTip® columns provide a perfect platform to automate this process. Also, antibody can be pre-immobilized and packed into the tip column, so the first half of the process is already completed.

Translating the manual IP process to the PhyTip® column is highlighted here by the work lead by Lilian Phu at Genentech.

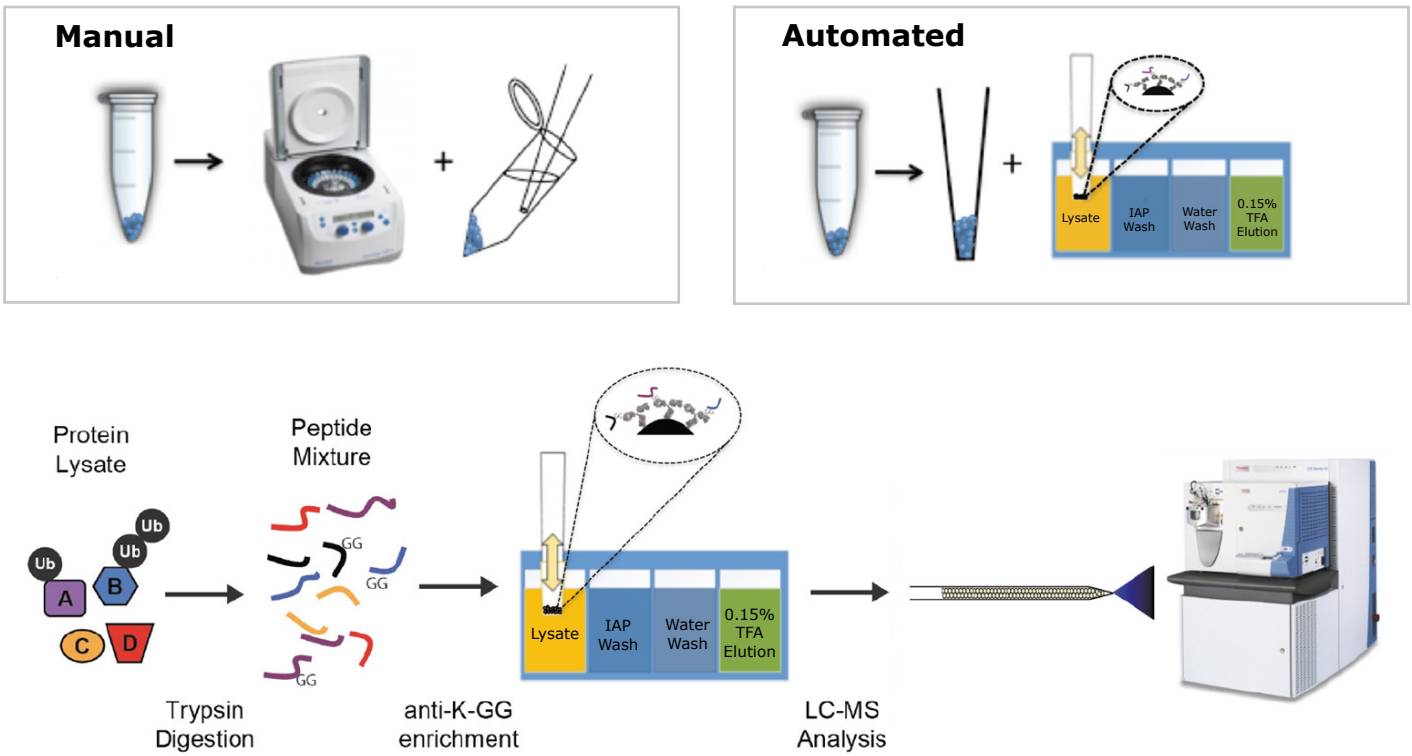


Figure 6. The immunoprecipitation process consists of multiple stages of capturing and washing both the antibody and the antigen. The process is fully a manual procedure. The process is cut in half by pre-packing antibody conjugated resin into the PhyTip® column. Furthermore, the rest of the process is automated by a robotic instrument.

Biotage®

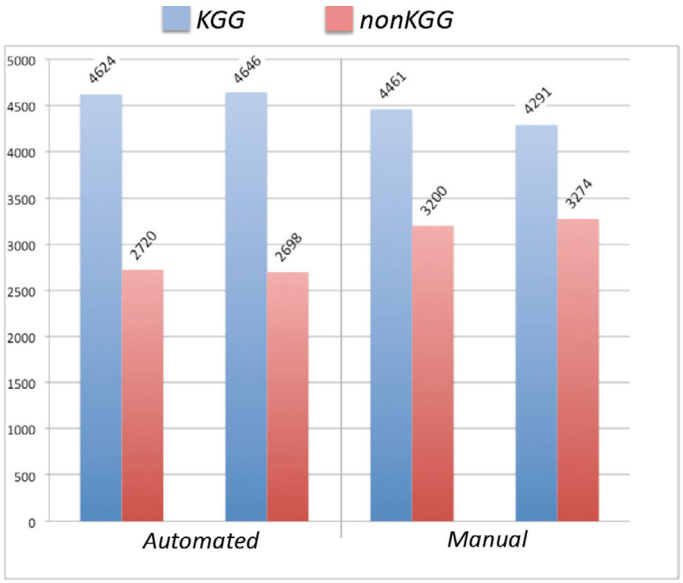


Figure 7. The immunoprecipitation method was used to isolate ubiquitin remnant motif (KGG) peptides from a complex mixture. These specific peptides were then analyzed by LC-MS/MS for KGG motif identity and for the level of non-specific contaminating peptides. When comparing the automated PhyTip® column process with the manual process, the automated procedure was more comparable. The automated process displayed more consistency and a lower background of non-specific peptides.

Conclusion

PhyTip® columns are a versatile platform that can be adapted to many different biomolecule purification applications. They can be used on all major automated liquid handling systems, enabling efficient set up of purification runs. Compared to alternative technologies PhyTip® columns offer easy automation, high sample recoveries and optimized concentrations through small elution volumes. This allows for high throughput solutions and purified biomolecule samples optimally prepared for following characterization steps.

Additional Application Data on PhyTip® Columns

Application Note: "MiniPrep PhyTip® Columns for Plasmid DNA Purification"

Application Note: "ProPlus PhyTip® Affinity Columns... The Antibody Purification System that is the Best of Protein A and Protein G"

Application Note: "Automated High Throughput Desalting and Buffer Exchange PhyTip® Columns"

Application Note: True parallel purification using PhyTip® column and Tecan Freedom EVO with Multichannel arm 96

Application Note: "High Performance Immunoprecipitation (HPIP)" Direct method

Application Note: "High Performance Immunoprecipitation (HPIP)" Indirect method

Application Note: "Optimization Strategies for High Performance Purification and Analysis of Recombinant Proteins with Micro Volume PhyTip® Columns and Caliper Life Sciences Automation"

Application Note: "Perform Resin Screening for Most Efficient Capture of Target Proteins"

EUROPE
Main Office: +46 18 565900
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 800 446 4752
Order Fax: +1 704 654 4917
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: +82 31 706 8500
Fax: +82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA
Tel: +91 11 45653772
india@biotage.com

Distributors in other regions are listed on www.biotage.com

Literature Number: PPS696

Biotage®