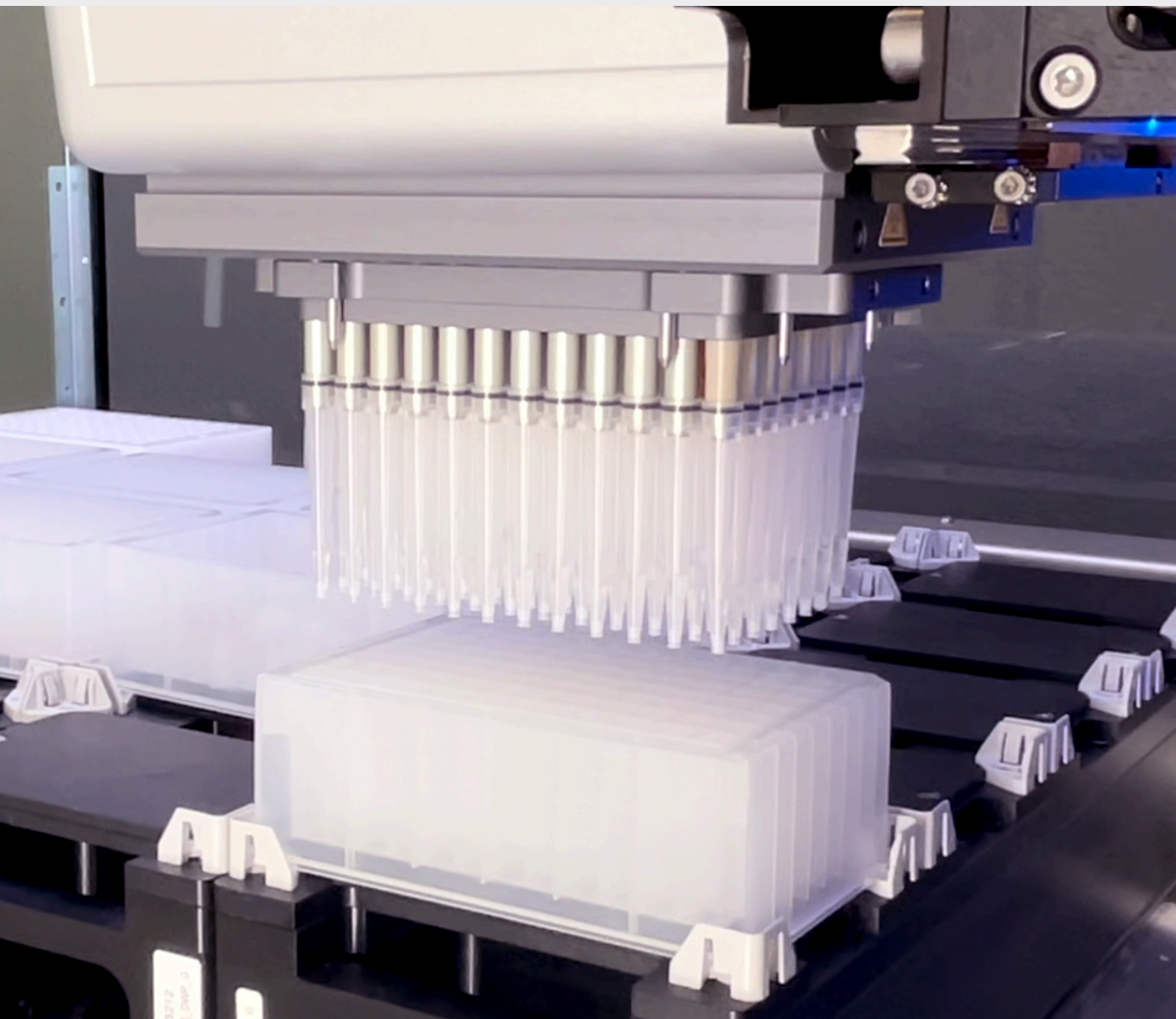


Biomolecule Purification with PhyTip® Columns for Hamilton® Robotics



High-Throughput Purification of Biomolecules with PhyTip® Columns

Hamilton® Robotics instruments combined with Biotage® PhyTip® columns provide a high-throughput, automated solution for purifying recombinant proteins, antibodies, AAVs, and plasmids. This combination enables efficient, automated protein purification for high-throughput lead screening, expression screening, and protein engineering.

Benefits

- » **Efficient Processing:** Purify 96 samples in 15-20 mins
- » **High Purity:** Mild chromatography conditions prevents denaturation resulting in high purity and recovery.
- » **Consistency and Scalability:** Ensure scalable and reproducible purification
- » **Versatile Sample Volume:** Ranging from 100 µL to 30 mL
- » **Range of column sizes:** 1 mL and 300 µL column sizes with resin bed volumes from 5 µL to 160 µL.

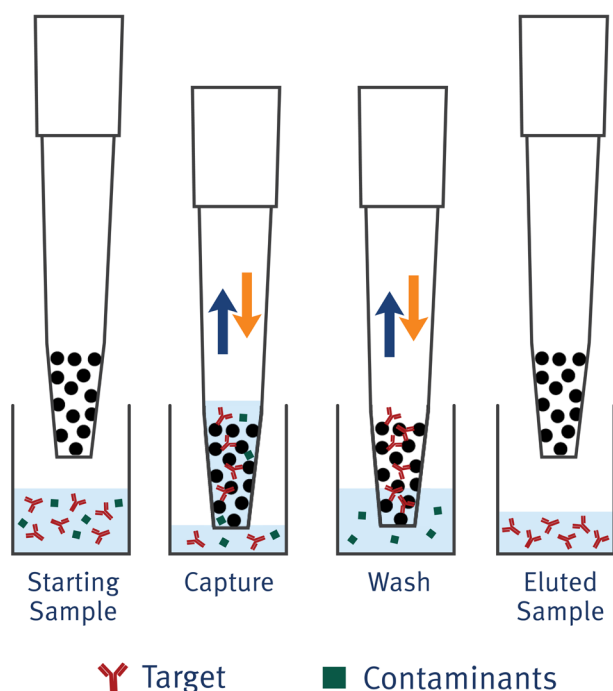
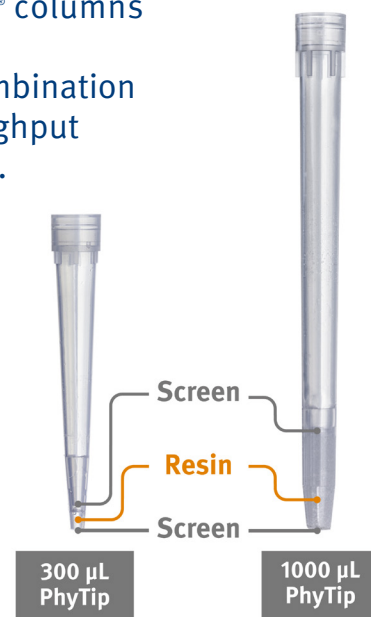


Figure 1. PhyTip® Columns purify samples through a repeated back-and-forth cycle of the sample through the packed resin bed, a process called dual flow chromatography. This process enables complete binding interactions during each purification step.

Efficient and Gentle Purification with PhyTip® Column Technology

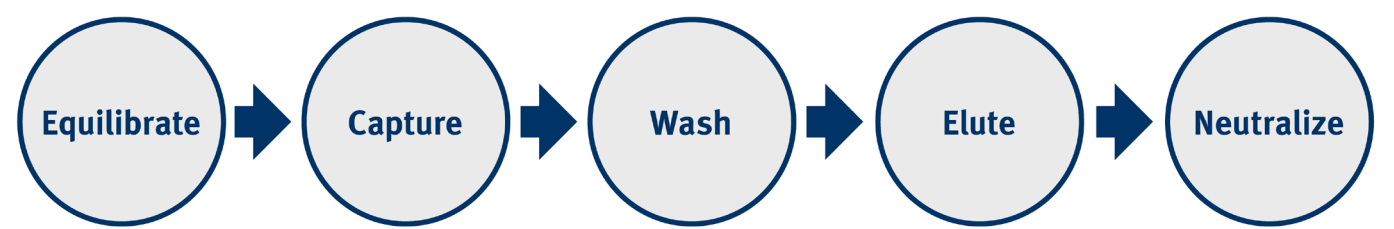
The advanced design of PhyTip® columns with packed resin bed supports the binding of the entire sample to a small resin bed volume. Fundamental chromatographic principles control the separation process, and the bi-directional flow facilitates interactions, leading to high loading equilibrium, irrespective of kinetic rate constants. The purified sample can be eluted in small volumes, resulting in a highly concentrated sample. Dual flow chromatography is a mild purification process yielding high levels of biologically active protein.

PhyTip® Columns can be used to purify biomolecules for basic research, drug discovery, and process development. Applications include, but are not limited to, plasmid purification, antibody purification, tagged recombinant protein purification, adeno-associated virus (AAV) purification, desalting, immunoprecipitation, and sample preparation for mass spectrometry.

The columns are available for Hamilton® Robotics instruments in column sizes of 300 µL and 1 mL, and in resin bed volumes from 5 µL to 160 µL for purification of microgram to milligram amounts of protein. Sample volumes can be varied between 100 µL and 30 mL. The PhyTip® Columns are available in a range of different chromatography resins from various suppliers. In addition, custom PhyTip® Columns are available upon request.

Antibody Purification Workflow for PhyTip® Columns on the Hamilton® Robotics

For antibody purification with PhyTip® Columns, the equilibration step conditions the columns for binding. During the capture step, back-and-forth flow captures the protein onto the column bed. The washing step removes contaminants while leaving the target protein on the column. In the elution step, a controlled low dead volume elutes the protein into small volumes, resulting in an efficient, high-concentration protein elution.



Purification of 96 IgG Samples Completed in Under 30 Minutes

To demonstrate the operation of the PhyTip® method on the Hamilton instrument, a full plate of hIgG samples was processed at once and analyzed by UV-vis spectrophotometry. ProPlus PhyTip® columns with a 20 µL resin bed size were used for this evaluation, using hIgG samples spiked into CHO media to represent a typical laboratory application of PhyTip® columns. The deck setup, parameter selection (page 4), and the PhyTip® purification were completed in under 30 minutes. Consistent recovery was achieved with a column volume (CV) of 4% across the 96 samples. PhyTip® columns, when paired with the advanced automation of Hamilton Robotics, provide a reliable and efficient solution for day-to-day purification workflows.

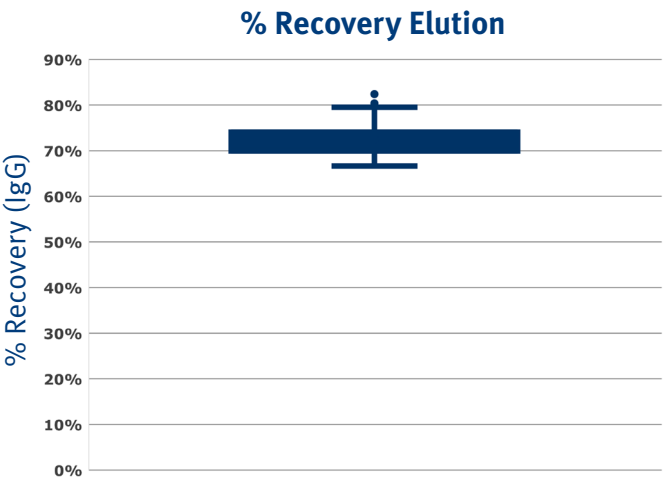


Table 1. Percent recovery of human IgG protein across 96 samples on the Hamilton® STAR liquid handler.

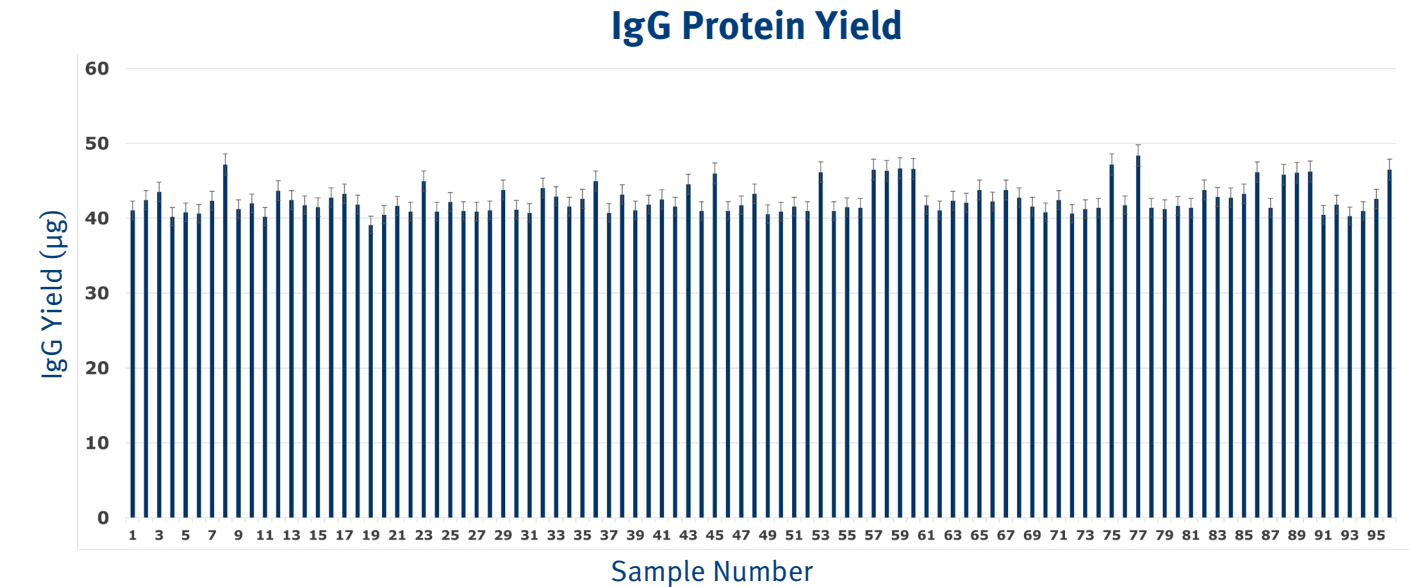
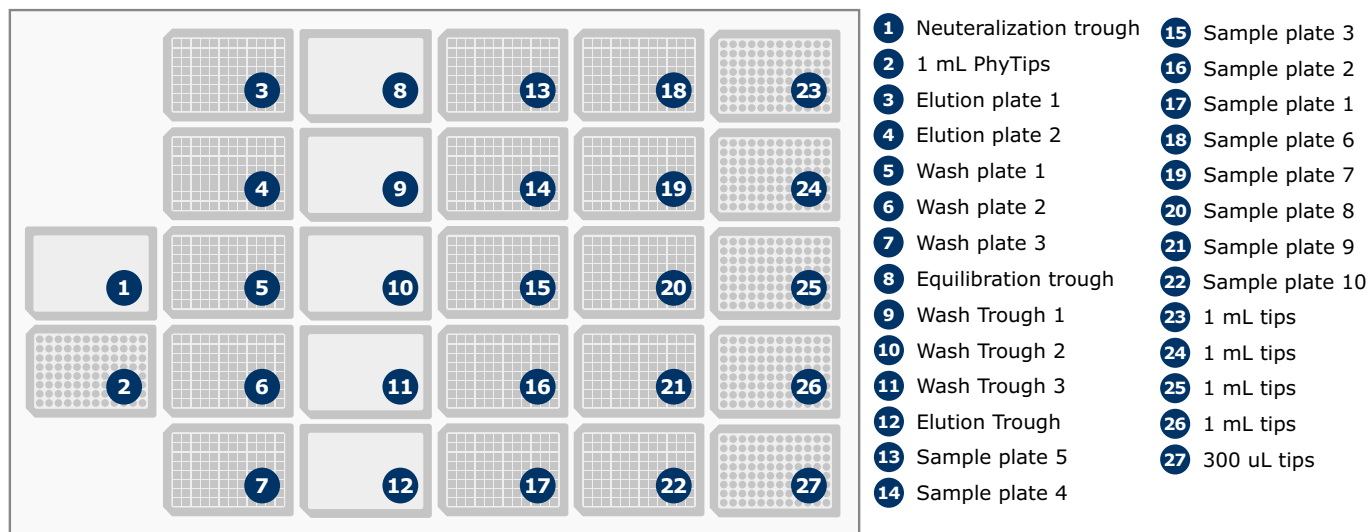


Table 2. IgG protein yield with a 4% CV across 96 samples on the Hamilton® STAR liquid handler.

Optimizing Workflow: Hamilton Deck Layout for Automated Protocols

The deck layout and components are designed for automated affinity or plasmid purification protocols. The method can accommodate up to 10 sample plates, 3 wash plates, and 2 elution plates based on your workflow requirements.



User-Friendly and Customizable Method

A fully automated, Hamilton®-validated method co-developed with Biotage® is available to use with PhyTip® columns. This method features a graphical user interface and requires no programming. It is fully customizable for optimizing processes involving 1-96 samples, resin type, PhyTip® column volume, mixing volumes, and the number of cycles for equilibration, capture, wash, elution, and neutralization.

Protein Purification

Resin Protein A/Protein G

PhyTip and Resin Volume 1mL, 20uL

Number of Samples 1

	Mix Volume	Number of Mix Cycles	Aspirate Flow Rate	Dispense Flow Rate
☒ Equilibration	200	1	Medium	Medium
Sample Capture Aliquot Sample	100	1	Medium	Medium
☒ Wash 1	10	2	Medium	Medium
☒ Wash 2	10	2	Medium	Medium
☒ Wash 3	10	2	Medium	Medium
☒ Elution ☒ Elution 2	50	1	Medium	Medium
☒ Sample Neutralization	15			

HAMILTON

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PhyTip® Columns

Antibody Purification

PhyTip® columns are available in a range of affinity media types that bind selectively to various part of the antibody structure. These columns are designed for purifying IgG antibodies, as well as other subtypes and fragments.

Our standard product portfolio includes resins such as Protein A, Protein G, and affinity resins specifically designed for binding antibody fragments, including renowned brands like MabSelect SuRe™ from Cytiva and CaptureSelect® from Thermo Fisher Scientific.

Tagged Recombinant Protein Purification

Several PhyTip® columns are available with affinity medias that bind His-tagged, Biotin-tagged and GST-tagged recombinant proteins.

Resins available in the standard product portfolio are IMAC affinity resins, Glutathione affinity resins and Streptavidin affinity resins.

Adeno-Associated Virus (AAV) Purification

Affinity chromatography purification can effectively remove impurities such as plasmid DNA and host cell RNA/DNA in AAV purification.

Plasmid Purification

Plasmids are the common starting material for transient transfection of recombinant proteins, mRNA and viral vectors. PhyTip® columns are available for high-throughput purification of plasmids.

Ion Exchange Chromatography

PhyTip® columns are available with different ion exchange chromatography resins, including strong anion exchange resins, weak anion exchange resins, strong cations exchange resins and weak cation exchange resins.

Gel Filtration/Desalting

Gel filtration PhyTip® columns packed with size exclusion chromatography resin are ideal for desalting protein samples and for buffer exchange. Desalting by gel filtration PhyTip® columns can be set up as a secondary purification step after affinity purification.

Reversed Phase Chromatography

Reversed Phase PhyTip® columns are used for automated desalting or cleanup of protein and peptide samples before mass spectrometry analysis. Reversed Phase PhyTip® columns bind a wide range of protein and polypeptide sizes.

Normal Phase Chromatography

Sample processing using Normal Phase PhyTip® columns through dual flow chromatography results in targeted enrichment of polar compounds, such as oligosaccharides. Normal Phase PhyTip® columns offer the optimal commercial solution for high-throughput oligosaccharide purification, and are particularly useful for applications such as oligosaccharide mapping and glycoprotein identification.

Custom Packed PhyTip® Columns

Biotage provides PhyTip® columns packed with diverse selection of resins for purifying various types of proteins. If our standard products do not meet your specific requirements, we also offer a custom packing services. Leveraging our extensive expertise in PhyTip® column packing, we can accommodate virtually any affinity media, silica, or resin with particle sizes $\geq 40 \mu\text{m}$ in the PhyTip® column format.

For more details, please visit:

www.biotage.com

Tip volumes (µL)	300		1000				
Resin volumes (µL)	5	20	10	20	40	80	160

Antibody Binding PhyTip® Columns

Part Number	Description	Qty.
Protein A Affinity Resin*		
PTH-93-05-01	300 µL PhyTip® column, 5 µL Protein A resin	96
PTH-93-10-01	300 µL PhyTip® column, 10 µL Protein A resin	96
PTH-93-20-01	300 µL PhyTip® column, 20 µL Protein A resin	96
PTH-91-10-01	1 mL PhyTip® column, 10 µL Protein A resin	96
PTH-91-20-01	1 mL PhyTip® column, 20 µL Protein A resin	96
PTH-91-40-01	1 mL PhyTip® column, 40 µL Protein A resin	96
PTH-91-80-01	1 mL PhyTip® column, 80 µL Protein A resin	96
PTH-91-16-01	1 mL PhyTip® column, 160 µL Protein A resin	96
Protein G Affinity Resin*		
PTH-93-05-02	300 µL PhyTip® column, 5 µL Protein G resin	96
PTH-93-10-02	300 µL PhyTip® column, 10 µL Protein G resin	96
PTH-93-20-02	300 µL PhyTip® column, 20 µL Protein G resin	96
PTH-91-10-02	1 mL PhyTip® column, 10 µL Protein G resin	96
PTH-91-20-02	1 mL PhyTip® column, 20 µL Protein G resin	96
PTH-91-40-02	1 mL PhyTip® column, 40 µL Protein G resin	96
PTH-91-80-02	1 mL PhyTip® column, 80 µL Protein G resin	96
PTH-91-16-02	1 mL PhyTip® column, 160 µL Protein G resin	96
ProPlus (MabSelect SuRe®) Affinity Resin*		
PTH-93-05-07	300 µL PhyTip® column, 5 µL ProPlus resin	96
PTH-93-10-07	300 µL PhyTip® column, 10 µL ProPlus resin	96
PTH-93-20-07	300 µL PhyTip® column, 20 µL ProPlus resin	96
PTH-91-10-07	1 mL PhyTip® column, 10 µL ProPlus resin	96
PTH-91-20-07	1 mL PhyTip® column, 20 µL ProPlus resin	96
PTH-91-40-07	1 mL PhyTip® column, 40 µL ProPlus resin	96
PTH-91-80-07	1 mL PhyTip® column, 80 µL ProPlus resin	96
PTH-91-16-07	1 mL PhyTip® column, 160 µL ProPlus resin	96
ProPlus LX (MabSelect SuRe® LX) Affinity Resin*		
PTH-93-05-26	300 µL PhyTip® column, 5 µL ProPlus LX resin	96
PTH-93-20-26	300 µL PhyTip® column, 20 µL ProPlus LX resin	96
PTH-91-10-26	1 mL PhyTip® column, 10 µL ProPlus LX resin	96
PTH-91-20-26	1 mL PhyTip® column, 20 µL ProPlus LX resin	96
PTH-91-40-26	1 mL PhyTip® column, 40 µL ProPlus LX resin	96
PTH-91-80-26	1 mL PhyTip® column, 80 µL ProPlus LX resin	96
PTH-91-16-26	1 mL PhyTip® column, 160 µL ProPlus LX resin	96
CaptureSelect® Affinity Resins		
PTH-93-05-30	300 µL PhyTip® column, 5 µL POROS CaptureSelect® IgM resin	96
PTH-93-10-30	300 µL PhyTip® column, 10 µL POROS CaptureSelect® IgM resin	96
PTH-93-20-30	300 µL PhyTip® column, 20 µL POROS CaptureSelect® IgM resin	96
PTH-91-10-30	1 mL PhyTip® column, 10 µL POROS CaptureSelect® IgM resin	96
PTH-91-20-30	1 mL PhyTip® column, 20 µL POROS CaptureSelect® IgM resin	96

*Buffer kit available

Part Number	Description	Qty.
PTH-93-05-32	300 µL PhyTip® column, 5 µL CaptureSelect® FcXL resin	96
PTH-93-20-32	300 µL PhyTip® column, 20 µL CaptureSelect® FcXL resin	96
PTH-91-10-32	1 mL PhyTip® column, 10 µL CaptureSelect® FcXL resin	96
PTH-91-20-32	1 mL PhyTip® column, 20 µL CaptureSelect® FcXL resin	96
PTH-93-05-24	300 µL PhyTip® column, 5 µL CaptureSelect® IgG-Fc (ms) resin	96
PTH-93-10-24	300 µL PhyTip® column, 10 µL CaptureSelect® IgG-Fc (ms) resin	96
PTH-93-20-24	300 µL PhyTip® column, 20 µL CaptureSelect® IgG-Fc (ms) resin	96
PTH-91-10-24	1 mL PhyTip® column, 10 µL CaptureSelect® IgG-Fc (ms) resin	96
PTH-91-20-24	1 mL PhyTip® column, 20 µL CaptureSelect® IgG-Fc (ms) resin	96
PTH-93-05-31	300 µL PhyTip® column, 5 µL CaptureSelect® KappaXL resin	96
PTH-93-10-31	300 µL PhyTip® column, 10 µL CaptureSelect® KappaXL resin	96
PTH-93-20-31	300 µL PhyTip® column, 20 µL CaptureSelect® KappaXL resin	96
PTH-91-10-31	1 mL PhyTip® column, 10 µL CaptureSelect® KappaXL resin	96
PTH-91-20-31	1 mL PhyTip® column, 20 µL CaptureSelect® KappaXL resin	96
PTH-93-05-35	300 µL PhyTip® column, 5 µL CaptureSelect® LambdaXP resin	96
PTH-93-10-35	300 µL PhyTip® column, 10 µL CaptureSelect® LambdaXP resin	96
PTH-93-20-35	300 µL PhyTip® column, 20 µL CaptureSelect® LambdaXP resin	96
PTH-91-10-35	1 mL PhyTip® column, 10 µL CaptureSelect® LambdaXP resin	96
PTH-91-20-35	1 mL PhyTip® column, 20 µL CaptureSelect® LambdaXP resin	96

Recombinant Protein PhyTip® Columns

Part Number	Description	Qty.
IMAC Affinity Resin*		
PTH-93-05-03	300 µL PhyTip® column, 5 µL IMAC resin	96
PTH-93-10-03	300 µL PhyTip® column, 10 µL IMAC resin	96
PTH-93-20-03	300 µL PhyTip® column, 20 µL IMAC resin	96
PTH-91-10-03	1 mL PhyTip® column, 10 µL IMAC resin	96
PTH-91-20-03	1 mL PhyTip® column, 20 µL IMAC resin	96
PTH-91-40-03	1 mL PhyTip® column, 40 µL IMAC resin	96
PTH-91-80-03	1 mL PhyTip® column, 80 µL IMAC resin	96
PTH-91-16-03	1 mL PhyTip® column, 160 µL IMAC resin	96

Part Number	Description	Qty.
Glutathione Affinity Resin*		
PTH-93-05-04	300 µL PhyTip® column, 5 µL Glutathione resin	96
PTH-93-10-04	300 µL PhyTip® column, 10 µL Glutathione resin	96
PTH-93-20-04	300 µL PhyTip® column, 20 µL Glutathione resin	96
PTH-91-10-04	1 mL PhyTip® column, 10 µL Glutathione resin	96
PTH-91-20-04	1 mL PhyTip® column, 20 µL Glutathione resin	96
PTH-91-40-04	1 mL PhyTip® column, 40 µL Glutathione resin	96
PTH-91-80-04	1 mL PhyTip® column, 80 µL Glutathione resin	96
PTH-91-16-04	1 mL PhyTip® column, 160 µL Glutathione resin	96

Streptavidin Affinity Resin

PTH-93-05-05	300 µL PhyTip® column, 5 µL Streptavidin resin	96
PTH-93-10-05	300 µL PhyTip® column, 10 µL Streptavidin resin	96
PTH-93-20-05	300 µL PhyTip® column, 20 µL Streptavidin resin	96
PTH-91-10-05	1 mL PhyTip® column, 10 µL Streptavidin resin	96
PTH-91-20-05	1 mL PhyTip® column, 20 µL Streptavidin resin	96
PTH-91-40-05	1 mL PhyTip® column, 40 µL Streptavidin resin	96
PTH-91-80-05	1 mL PhyTip® column, 80 µL Streptavidin resin	96
PTH-91-16-05	1 mL PhyTip® column, 160 µL Streptavidin resin	96

Chromatography PhyTip® Columns

Part Number	Description	Qty.
Strong Anion Resin		
PTH-93-05-11	300 µL PhyTip® column, 5 µL Strong Anion resin	96
PTH-93-10-11	300 µL PhyTip® column, 10 µL Strong Anion resin	96
PTH-93-20-11	300 µL PhyTip® column, 20 µL Strong Anion resin	96
PTH-91-10-11	1 mL PhyTip® column, 10 µL Strong Anion resin	96
PTH-91-20-11	1 mL PhyTip® column, 20 µL Strong Anion resin	96
PTH-91-40-11	1 mL PhyTip® column, 40 µL Strong Anion resin	96
PTH-91-80-11	1 mL PhyTip® column, 80 µL Strong Anion resin	96
PTH-91-16-11	1 mL PhyTip® column, 160 µL Strong Anion resin	96

Strong Cation Resin

PTH-93-05-13	300 µL PhyTip® column, 5 µL Strong Cation resin	96
PTH-93-10-13	300 µL PhyTip® column, 10 µL Strong Cation resin	96
PTH-93-20-13	300 µL PhyTip® column, 20 µL Strong Cation resin	96
PTH-91-10-13	1 mL PhyTip® column, 10 µL Strong Cation resin	96
PTH-91-20-13	1 mL PhyTip® column, 20 µL Strong Cation resin	96
PTH-91-40-13	1 mL PhyTip® column, 40 µL Strong Cation resin	96
PTH-91-80-13	1 mL PhyTip® column, 80 µL Strong Cation resin	96
PTH-91-16-13	1 mL PhyTip® column, 160 µL Strong Cation resin	96

Weak Anion Resin

PTH-93-05-12	300 µL PhyTip® column, 5 µL Weak Anion resin	96
PTH-93-10-12	300 µL PhyTip® column, 10 µL Weak Anion resin	96
PTH-93-20-12	300 µL PhyTip® column, 20 µL Weak Anion resin	96
PTH-91-10-12	1 mL PhyTip® column, 10 µL Weak Anion resin	96
PTH-91-20-12	1 mL PhyTip® column, 20 µL Weak Anion resin	96
PTH-91-40-12	1 mL PhyTip® column, 40 µL Weak Anion resin	96
PTH-91-80-12	1 mL PhyTip® column, 80 µL Weak Anion resin	96
PTH-91-16-12	1 mL PhyTip® column, 160 µL Weak Anion resin	96

Part Number	Description	Qty.
Weak Cation Resin		
PTH-93-05-14	300 µL PhyTip® column, 5 µL Weak Cation resin	96
PTH-93-10-14	300 µL PhyTip® column, 10 µL Weak Cation resin	96
PTH-93-20-14	300 µL PhyTip® column, 20 µL Weak Cation resin	96
PTH-91-10-14	1 mL PhyTip® column, 10 µL Weak Cation resin	96
PTH-91-20-14	1 mL PhyTip® column, 20 µL Weak Cation resin	96
PTH-91-40-14	1 mL PhyTip® column, 40 µL Weak Cation resin	96
PTH-91-80-14	1 mL PhyTip® column, 80 µL Weak Cation resin	96
PTH-91-16-14	1 mL PhyTip® column, 160 µL Weak Cation resin	96

Reversed Phase Resin C18 90Å Pore Size

PTH-93-05-18	300 µL PhyTip® column, 5 µL C18 90Å resin	96
PTH-93-10-18	300 µL PhyTip® column, 10 µL C18 90Å resin	96
PTH-93-20-18	300 µL PhyTip® column, 20 µL C18 90Å resin	96
PTH-91-10-18	1 mL PhyTip® column, 10 µL C18 90Å resin	96
PTH-91-20-18	1 mL PhyTip® column, 20 µL C18 90Å resin	96
PTH-91-40-18	1 mL PhyTip® column, 40 µL C18 90Å resin	96
PTH-91-80-18	1 mL PhyTip® column, 80 µL C18 90Å resin	96
PTH-91-16-18	1 mL PhyTip® column, 160 µL C18 90Å resin	96

Reversed Phase Resin C18 300Å Pore Size

PTH-93-05-19	300 µL PhyTip® column, 5 µL C18 300Å resin	96
PTH-93-10-19	300 µL PhyTip® column, 10 µL C18 300Å resin	96
PTH-93-20-19	300 µL PhyTip® column, 20 µL C18 300Å resin	96
PTH-91-10-19	1 mL PhyTip® column, 10 µL C18 300Å resin	96
PTH-91-20-19	1 mL PhyTip® column, 20 µL C18 300Å resin	96
PTH-91-40-19	1 mL PhyTip® column, 40 µL C18 300Å resin	96
PTH-91-80-19	1 mL PhyTip® column, 80 µL C18 300Å resin	96
PTH-91-16-19	1 mL PhyTip® column, 160 µL C18 300Å resin	96

Reversed Phase Resin C4 300Å Pore Size

PTH-93-05-52	300 µL PhyTip® column, 5 µL C4 300Å resin	96
PTH-93-10-52	300 µL PhyTip® column, 10 µL C4 300Å resin	96
PTH-93-20-52	300 µL PhyTip® column, 20 µL C4 300Å resin	96
PTH-91-10-52	1 mL PhyTip® column, 10 µL C4 300Å resin	96
PTH-91-20-52	1 mL PhyTip® column, 20 µL C4 300Å resin	96
PTH-91-40-52	1 mL PhyTip® column, 40 µL C4 300Å resin	96
PTH-91-80-52	1 mL PhyTip® column, 80 µL C4 300Å resin	96
PTH-91-16-52	1 mL PhyTip® column, 160 µL C4 300Å resin	96

Normal Phase 1 Resin

PTH-93-05-09	300 µL PhyTip® column, 5 µL Normal Phase 1 resin	96
PTH-93-10-09	300 µL PhyTip® column, 10 µL Normal Phase 1 resin	96
PTH-93-20-09	300 µL PhyTip® column, 20 µL Normal Phase 1 resin	96
PTH-91-10-09	1 mL PhyTip® column, 10 µL Normal Phase 1 resin	96
PTH-91-20-09	1 mL PhyTip® column, 20 µL Normal Phase 1 resin	96
PTH-91-40-09	1 mL PhyTip® column, 40 µL Normal Phase 1 resin	96
PTH-91-80-09	1 mL PhyTip® column, 80 µL Normal Phase 1 resin	96
PTH-91-16-09	1 mL PhyTip® column, 160 µL Normal Phase 1 resin	96

AAV PhyTip® Columns

Part Number	Description	Qty.
CaptureSelect™ AAVX Resin		
PTH-93-05-33	300 µL PhyTip® column, 5 µL CaptureSelect® AAVX resin	96
PTH-93-10-33	300 µL PhyTip® column, 10 µL CaptureSelect® AAVX resin	96
PTH-93-20-33	300 µL PhyTip® column, 20 µL CaptureSelect® AAVX resin	96
PTH-91-10-33	1 mL PhyTip® column, 10 µL CaptureSelect® AAVX resin	96
PTH-91-20-33	1 mL PhyTip® column, 20 µL CaptureSelect® AAVX resin	96
PTH-91-40-33	1 mL PhyTip® column, 40 µL CaptureSelect® AAVX resin	96
PTH-91-80-33	1 mL PhyTip® column, 80 µL CaptureSelect® AAVX resin	96
PTH-91-16-33	1 mL PhyTip® column, 160 µL CaptureSelect® AAVX resin	96

Gel Filtration/Desalting PhyTip® Columns

Part Number	Description	Qty.
5K Desalting Resin		
PDR-91-20-06	1 mL PhyTip® column, 200 µL 5K desalting resin	96
PDR-91-60-06	1 mL PhyTip® column, 600 µL 5K desalting resin	96
30K Desalting Resin		
PDR-91-20-17	1 mL PhyTip® column, 200 µL 30K desalting resin	96
PDR-91-60-17	1 mL PhyTip® column, 600 µL 30K desalting resin	96

Plasmid Purification PhyTip® Columns

Part Number	Description	Qty.
Plasmid Miniprep		
DPH-91-80-70	1 mL PhyTip® column, 80 µL Miniprep silica and buffers for Plasmid Miniprep	96

Accessories

Part Number	Description	Qty.
DAH-12-000-02	PhyTip column transfer tool for Hamilton instrument	1
DAH-96-000-01	Drying station adaptor for Lysate Direct PhyTip columns for Hamilton system. Necessary for automated plasmid MiniPrep	1
DAH-96-000-02	PhyTip column transfer tool. Allows user to manually transfer 12 Hamilton tips at a time from one tip rack to another.	1
PHF-96-200-01	Desalting Adaptor Block for 200 µL Desalting columns for robotic platforms with gripper arm	1
PHF-96-600-01	Desalting Adaptor Block for 600 µL Desalting columns for robotic platforms with gripper arm	1

Literature Number: PPS752

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