

PhyTip® Columns Containing CaptureSelect® Resins for Purification of Antibodies and Antibody Fragments

Introduction

The Biotage PhyTip® columns are the proven technology of choice for the purification of recombinant proteins and antibodies when requiring robust high quality reproducible separations from small-volume samples while maintaining high protein activity. The innovative methods developed for processing PhyTip columns result in equilibrium binding and elution and have virtually no dead volume allowing for efficient capture and the highest concentration of eluted active proteins possible with results that are scalable to manufacturing volumes (Figure 1). The columns are supported by many liquid handling systems for ease of integration and high throughput.

Biotage is excited to offer PhyTip columns containing the CaptureSelect® resins from BAC BV. As the new spectrum of antibody formats evolves the current affinity ligands cannot always provide the binding and elution specificity demanded. CaptureSelect products are affinity ligands created by a proprietary technology based on Camelid derived single domain antibody fragments. These products possess a combination of unique properties such as stability, affinity, and selectivity that provides competitive benefits in terms of reduced cost of purification, higher quality product, reduced development and increased flexibility in the purification process to eliminate uncertainty, streamline protocols and ensure confidence that the final product is the intended antibody species. This is achieved through specific affinities to novel antibody domains (Figure 2).

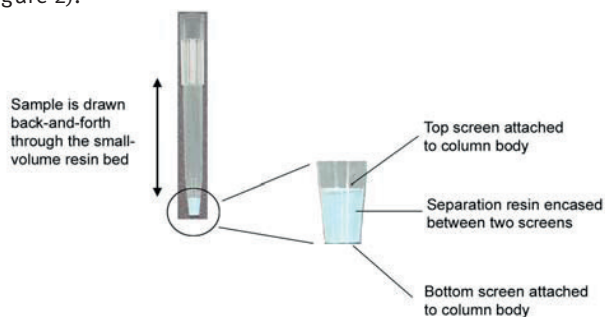


Figure 1. PhyTip® column containing 40 µL of resin.

Through Biotage custom packing program, researchers get the benefits of using the CaptureSelect resins in the PhyTip column format. In this note, we highlight data achieved with this dual platform on BSA depletion with CaptureSelect Multi Species Albumin and hlgG capture with either Fc specific CaptureSelect Human Fc affinity matrix or Kappa chain specific CaptureSelect Fab Kappa matrix, just a few of the resin offerings from BAC.

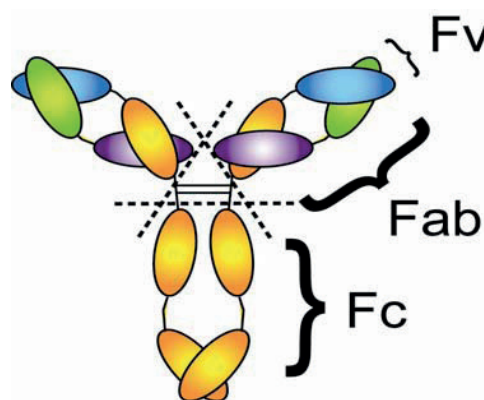


Figure 2. CaptureSelect Antibody Toolbox purification ligands are specific to novel antibody domains.

Resin

- » Human Fc affinity matrix:
For all subclasses of Human IgG
- » Multi Species affinity matrix: For IgG from multiple species
- » Human Fab kappa affinity matrix:
For all Human kappa Fab fragments
- » Human Fab lambda affinity matrix:
For all Human lambda Fab fragments
- » IgA affinity matrix:
For Human IgA
- » IgM affinity matrix:
For Human and Murine IgM
- » IgG4 affinity matrix:
For Human monoclonal and polyclonal IgG4's

Materials and Methods

CaptureSelect Multi Species Albumin was packed into 1 mL PhyTip® columns of 10, 20 and 40 µL resin bed sizes. Using the MEA personal purification system from Biotage, these columns were tested, in triplicate, for removal of bovine serum albumin (SeraCare Life Sciences) from PBS buffer. Columns were equilibrated with 1 cycle of back and forth flow in 500 µL PBS using a flow rate of 0.5 mL/min. and 20 second pauses after each aspirate and expel steps. The equilibrated columns were asked to capture BSA from 500 µL PBS, 0.05% Tween 20 spiked with BSA to a final concentration of 0.84 mg/mL by performing four back-and-forth capture cycles at 0.5 mL/min using 20 second pauses. The flow through was measured for absorbance at 280 nm using a NanoDrop UV Spectrometer. 1 mL PhyTip columns containing 10, 20 or 40 µL of either CaptureSelect Human Fc affinity matrix or CaptureSelect Fab Kappa affinity matrix resins was used to purify 500 µL PBS, 0.05% Tween 20 samples spiked with hlgG to a final concentration of 0.01 mg/mL. Columns were equilibrated with 1 cycle of back and forth flow in 1 mL PBS using a flow rate of 1 mL/min and 20 second pauses after each aspirate and expel steps. The equilibrated columns captured 500 µL hlgG samples by performing 4 back-and-forth capture cycles at 1 mL/min using 20 second pauses. Columns were washed in 500 µL PBS for 1 cycle at 1 mL/min and repeated for 500 µL 140mM NaCl. Elution was performed in 3 times the column resin bed volume of 0.2mM phosphate pH 3.0, 140mM NaCl using 4 cycles at 1 mL/min and 20 second pauses. Samples were neutralized with ¼ the elution buffer volume using 1M Tris pH 9.0. 15 µL of the neutralized elution was adjusted to 120µL with PBS, 0.05% Tween 20 and 80 µL was injected into an HPLC ion pairing reverse phase column. The area under the peaks corresponding to hlgG were integrated and fitted to a standard curve to determine the mass injected. Alternatively, samples were analyzed by SDS PAGE.

Table 1. Binding Capacity of CaptureSelect Multi Species Albumin.

Resin Bed Volume	Binding Capacity (mg BSA/mL resin)	Average Capacity (mg BSA/mL resin)
10 µL - PhyTip column 1	7.5	
10 µL - PhyTip column 2	4.5	
10 µL - PhyTip column 3	8.5	6.8
20 µL - PhyTip column 1	7.8	
20 µL - PhyTip column 2	7.8	
20 µL - PhyTip column 3	7.8	7.8
40 µL - PhyTip column 1	5.5	
40 µL - PhyTip column 2	5.4	
40 µL - PhyTip column 3	6.5	5.8

Results

Bovine Serum Albumin Depletion

For complex biological samples such as serum, high concentrations of albumin may interfere with the reliability of certain assays. 1 mL PhyTip columns were used to determine the dynamic binding capacity of CaptureSelect Multi Species Albumin. 500 µL of PBS, 0.05% Tween 20 were spiked with bovine serum albumin (BSA) to a final concentration of about 0.8 mg/mL. PhyTip columns containing 10, 20 or 40 µL of CaptureSelect Multi Species Albumin was used to deplete the sample of BSA. The sample flow through was measured for absorbance at 280 nm and a binding capacity of 7 µg/µL resin was determined (Table 1). The PhyTip columns are sufficient to deplete samples of BSA and is highly reproducible.

Human IgG Capacity

For purification of Human immunoglobulin G (hlgG), BAC offers several CaptureSelect resins. CaptureSelect Human Fc affinity-matrix and CaptureSelect Fab Kappa affinity matrix resins were measured for recovery of hlgG. The system was stressed by simulating a low expressing IgG sample that a researcher may encounter. The PhyTip columns recovered 5–10% of the IgG from samples at a starting concentration of 0.01mg/mL hlgG (Figure 3 and Table 2).

The dynamic binding capacity is proportional to the starting sample concentration. When purifying samples of higher concentration, 1.0 mg/mL hlgG, the dynamic binding capacity is around 3.5 mg/mL (Table 3). Purifying IgG from high concentration samples such as serum results in a binding capacity of 15 mg/mL. The PhyTip Column format is highly versatile and the information used to determine purification conditions on small samples is scalable to large-scale purifications.

Table 2. PhyTip® Columns Containing 5 mL Human Fc Resin Capture of 200 µL 10 mg/mL hIgG Samples.

	Uncaptured hIG (µg)	% Captured	Capacity (mg/mL resin)	Average Capacity (mg/mL resin)
Fc-1	102.7	32	9.5	8.4
Fc-2	110.9	26	7.8	
Fc-3	110.7	26	7.9	

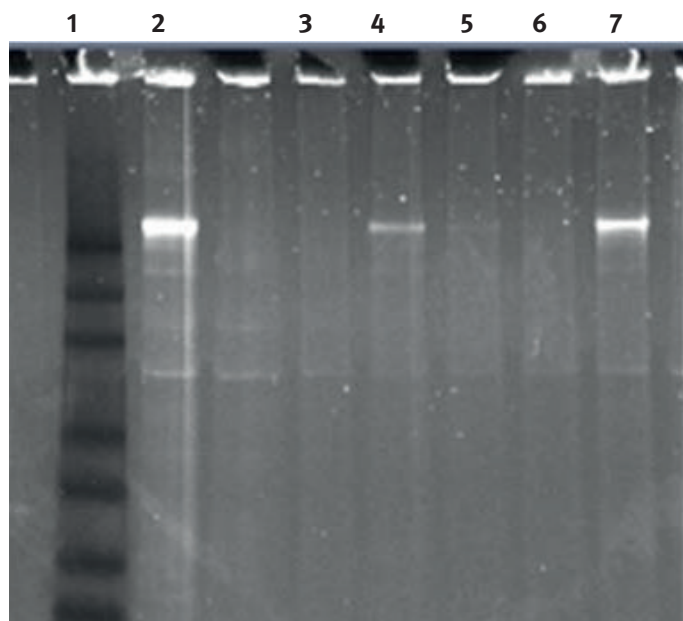


Figure 3. SDS-Page analysis of 500 µL 0.01 mg/mL IgG samples. Samples were processed by PhyTip columns containing 80 µL CaptureSelect Human Fab kappa affinity resin. The figure shows a picture of the gel taken after the gels were run, stained with coomassie blue, and destained. Lane 1 was loaded with a molecular weight marker. Starting sample was loaded in Lane 2. Lanes 3–7 represent aliquots from the various buffers after PhyTip column processing of the pre equilibration buffer (3), sample flow through (4), wash 1 (5), wash 2 (6) and elution (7), respectively.

Discussion

PhyTip® columns are the platform of choice for small-volume purifications of antibodies and recombinant proteins. The high performance obtained from these columns provides an unprecedented ability to scale-up purifications to large, manufacturing-scale columns. Small volume purifications allow researchers to make decisions earlier in their processes by providing data with which to make informed decisions. When screening for antibody interactions to interesting targets, the parallel, walk-away feature of the PhyTip columns means reduced labor and time costs.

The flexibility of the PhyTip columns is enhanced by the ability to utilize different extraction media. The affinity matrices available from BAC provide a novel solution for the purification of antibodies and antibody fragments. Having developed affinity ligands for specific antibody domains, BAC's CaptureSelect resins offer a great enhancement over traditional bacteria coat proteins. Affinity for the Fc constant regions of antibodies provides a clear advantage over Protein L resins, which are limited by interacting only with kappa light chains. Unlike Protein A, CaptureSelect Fc is able to bind all 4 human IgG subclasses.

When Purifying Fab fragments, combining CaptureSelect Kappa and CaptureSelect Lambda offers the only available product that purifies all Fab fragments. IgMs, which have traditionally been overlooked by researchers because of their large size and tendency to aggregate or become labile make them difficult to purify, are now suitable for study with true affinity-based capture technology.

The utility of performing small-volume purifications, with sufficient efficiency, is especially useful when samples are precious or of low abundance, such as patient serum samples. Specific applications such as serum profiling for diagnostics development can lead to early detection of disease. CaptureSelect resins with affinity for IgA are especially intriguing because IgAs are implicated as a biomarker for inflammatory disease and have been implicated in a role as an upper bowel transcytotic carrier molecule, which may be able to deliver drugs to specific regions. Together PhyNexus and BAC technologies offer new solutions to major bottlenecks in the bio-therapeutics and disease diagnostics process.

Table 3. Binding capacity of CaptureSelect resins of 1 mg/mL IgG samples.

	Binding Capacity (mg hIG/mL Resin)
Human Fab kappa	3.4
Human Fc	3.5

Conclusion

The combination of Biotage separation columns and BAC resins provide the most efficient and precise early stage evaluation for the spectrum of new developing antibody formats such as Fc fusions, Fab and FAb fragments, IgA's, IgM's, and IgG subclasses such as IgG4. The specificity of the BAC resins for binding and elution conditions make them optimal for many sensitive and specific applications and the PhyTip® columns provide an efficient platform to explore their optimization. The flexibility of the PhyTip columns allow for rapid data generation from small comparable resin volumes for screening binding and elution conditions to larger volumes with consistent yield for analytical assays. Results from initial experiments are scalable for predictable later stage manufacturing separations. Automating and miniaturizing the optimization process improves speed of data acquisition and data quality to avoid errors and limits valuable reagent use. Generating concentrated proteins from smaller volumes from more constructs earlier in the development process allows for additional analysis and subsequently better characterized bio-therapeutics in the pipeline.

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