WHITEPAPER

Advantages of Antibody-based Selectivity in the Purification of Next Generation Biologics

Antibody-based affinity chromatography using CaptureSelect™ affinity resins enables highly selective process-scale purification of biologic drugs at increased yields for reduced time to market

It has been almost 40 years since the first biologic drug reached the market.[1] That drug was human insulin, which was produced by cloning human genes into *E. coli* for protein expression at a scale suitable for therapeutic use. Today, biologics are expected to be the fastest-growing segment of the pharmaceutical industry, reaching a predicted worth of $1.2 trillion by 2030.[2]

Because biologic drugs are produced in living systems, it is critical that they are separated from potentially harmful contaminants such as host cell proteins, cell debris, and any other impurities before being dosed in man. However, established purification techniques employ lengthy protocols and often incur substantial product losses, leading many biologics manufacturers to seek a more viable alternative.

Antibody-based affinity chromatography is a preferred method of purification in next generation biologics manufacturing. Not only does it benefit from higher selectivity and greater yields than existing approaches, but it also involves fewer process steps which helps enable a faster time to market. CaptureSelect™ affinity resins from Thermo Fisher Scientific are designed to meet a wide variety of affinity chromatography needs and are easily integrated into biologics production workflows.

Non-affinity purification methods have inherent limitations

Biologics such as recombinant proteins and viral particles have traditionally been purified by size-exclusion chromatography, ion-exchange chromatography, or specialized filtration techniques. These processes exploit one or more physical properties of the biomolecule

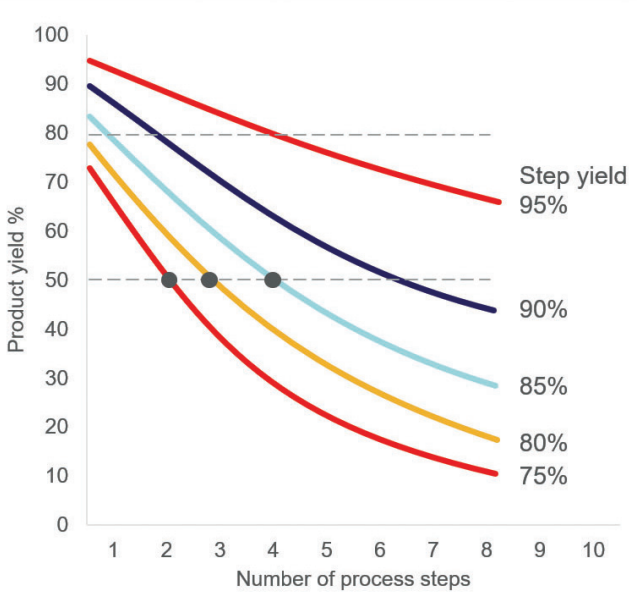

Figure 1. Each step in the purification process results in a yield loss. Even at higher step yields the overall product yield decreases rapidly with the increase in process steps. Optimizing the purification process to minimize the total number of steps is crucial to increase efficiency in the total process.

to separate it from any contaminants. Yet, although they achieve the desired purity, the large number of steps involved invariably compromises yields (Figure 1). In turn, this increases associated manufacturing costs.

In contrast, monoclonal antibodies have historically been purified by Protein A affinity chromatography. During this process, the immobilized *S. aureus* cell wall protein binds and captures antibodies via the Fc region. Despite being a robust and reliable technology, Protein A affinity chromatography runs the risk of antibodies aggregating in the slightly acidic conditions required for elution. A further limitation of this method is that it is unsuitable for newer antibody therapeutics where the Fc region is lacking (e.g., Fab fragments) or has been modified in some way (e.g., bispecific antibodies).

Antibody-based affinity chromatography provides superior purification

Antibody-based affinity chromatography, such as used in the CaptureSelect™ platform, was developed as a more robust alternative to existing methods for purifying biologics. Similarly to Protein A chromatography, it uses an immobilized affinity ligand to capture the target biomolecule from a complex solution. However, during antibody-based affinity chromatography, the affinity ligand comprises a single domain antibody in place of Protein A.

Unlike an IgG, that consists of two identical heavy chains and two identical light chains, each with an Fc domain at one end and a variable (antigen-binding) domain at the other, a single domain antibody comprises just one variable domain of a heavy chain (Figure 2). For this reason, it is sometimes referred to as a V_HH antibody.

Single domain antibodies are considerably smaller than IgGs, having a molecular weight of around 15 kDa compared to approximately 160 kDa. This feature, in combination with zero requirement for a light-chain complement to bind target molecules, means that single domain antibodies are more stable and easier to manufacture than full-length antibodies. This makes them an ideal tool for purifying biologics.

Other important characteristics of single domain antibodies underpinning their use for biologics purification include extremely high specificity and affinity for target biomolecules. They also benefit from a low toxicity profile and do not require animal-derived components during production since they can be manufactured in lower eukaryotes such as yeast. In addition, single domain antibodies demonstrate minimal lot-to-lot variability, providing exceptional consistency between production runs.

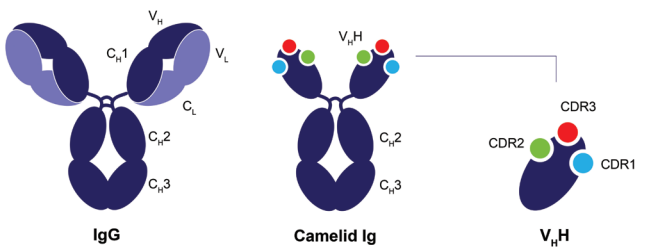

Figure 2. Comparison of an IgG antibody and a single domain (V_HH) antibody.

Because single domain antibodies are so versatile, antibody-based affinity chromatography can be used to purify almost any type of biologic, including recombinant proteins, viral particles, monoclonal antibodies and newer antibody therapeutics. Moreover, the highly specific nature of the binding interaction means fewer purification steps, resulting in improved yields and a high purity. This translates to shorter bioprocess development time and, ultimately, reduced time to market.

CaptureSelect affinity resins maximize the efficiency of capture chromatography

CaptureSelect™ technology is based on chromatography beads functionalized with single domain antibodies. These antibodies are manufactured in yeast for a product that is completely animal origin-free. During CaptureSelect™ purification, the target biomolecule binds the single domain antibodies immobilized on the beads. After this, any non-target biomolecules like host cell proteins and cell debris are washed away and the target is eluted.

Using CaptureSelect™ resins for purifying biologics provides many advantages. These not only include the high selectivity conferred by the single domain antibodies, but also the incredibly efficient clearance of any impurities. In addition, the high selectivity of affinity binding contributes to the overall viral reduction of the process, increasing the safety of the final product with respect to viral contamination.[3,4] Another important feature of CaptureSelect™ resins is that they allow for the use of mild elution conditions, an especially critical consideration when purifying new biotherapeutics whose function could otherwise be compromised by denaturation.

The CaptureSelect™ family currently comprises three main product groups — CaptureSelect™ antibody subdomain-specific affinity chromatography resins for purifying antibody therapeutics, CaptureSelect™ affinity matrices for purifying recombinant proteins (including protein biosimilars/biobetters), and a range of innovative cell and gene therapy purification solutions. Many additional products are in development to meet evolving needs within biologics production.

A five-step platform production process assures resin quality

CaptureSelect™ affinity resins are developed and produced via a standardized five-step platform process based on strict go/no-go principles that leverage customer feedback and testing. The process begins with selection or construction of a library of single domain antibodies (referred to as ligands) that is carefully tailored to the biologic of interest.

Next, a high-throughput screening step is performed to identify those ligands that best capture the target biomolecule. During screening, critical factors such as specificity, on- and off-rates of binding, and ligand stability are evaluated and ligands are additionally assessed for their ability to release the target under mild elution conditions.

CaptureSelect resins for purifying biologics

The CaptureSelect product family is designed to support a diverse range of purification requirements within biologics manufacturing. CaptureSelect resins are complemented by product specific ELISAs for analyzing ligand leachate from the column, cGMP-ready pre-packed column formats, and regulatory support files.

CaptureSelect purification of antibody therapeutics

CaptureSelect antibody subdomain-specific affinity chromatography resins are developed for the downstream purification of novel antibody therapeutics including monoclonal antibodies, bispecific monoclonal antibodies, Fab fragments, and Fc-fusion proteins.

They provide a superior alternative to Protein A resins for IgGs and Fc-fusion proteins exhibiting poor Protein A binding, and benefit from mild elution conditions for pH-sensitive antibody therapeutics.

Where CaptureSelect antibody subdomain-specific affinity chromatography resins are used for antibody fragment purification, the risk of co-purifying free light chains is eliminated. These resins enable single step purification of all antibody fragments, whether derived from the kappa or lambda family.

Products include resins for human CH1 domain binding, human C_L – kappa domain binding, and human CH3 domain binding.

CaptureSelect purification of recombinant proteins

CaptureSelect affinity matrices for purifying recombinant proteins are a unique portfolio of resins for the downstream purification of biosimilars, biobetters, and other types of recombinant protein biologics.

They retain the biological activity of the captured target by using mild elution conditions and provide highly efficient clearance of impurities to support regulatory filings.

Products include resins for purifying human follicle stimulating hormone (hFSH), human serum albumin, human chorionic gonadotropin (hCG), human growth hormone (hGH), human tissue plasminogen activator (tPA), human thyroid stimulating hormone (hTSH), and the widely used affinity tag E-P-E-A.

CaptureSelect purification of viral vectors for cell and gene therapies

CaptureSelect technology underpins several innovative chromatography solutions designed to improve the purification of cell and gene therapies based on viral vectors.

Combined with POROS™ beads that have a large pore structure to enhance biomolecule binding, these specialized affinity resins maximize process consistency, efficiency, and productivity for biologics that are known to present unique purification challenges.

Products include resins for purifying the adeno-associated virus (AAV) vectors AAVX, AAV9, and AAV8.

The third step in the platform production process involves manufacturing a small number of prototype resins by combining promising ligands with a chromatography backbone of choice. These are then used in a single-step test purification to determine how the ligand performs when immobilized on the resin and demonstrate the scalability of the final product.

Following this, two promising lead resins are tested at larger scale. This provides further opportunities to optimize the ligand density and backbone for chromatography and is performed under actual process conditions to establish which resin gives the best separation.

Finally, once the ligand and resin have been identified, the process is further validated and ancillary tools are developed. These include an enzyme-linked immunosorbent assay (ELISA) that can detect the affinity ligand in solution if it were to be stripped from the resin backbone during chromatography. Having access to such an immunoassay is especially valuable for manufacturers of biologic drugs, who will need to demonstrate that the affinity chromatography step is not inadvertently introducing unwelcome proteins into the mixture.[5]

The project is completed with compilation of a regulatory support file which contains all details of the ligand and resin manufacturing process. This includes, for example, data generated from toxicology studies and during testing of master cell banks/working cell banks, such as is required to file for regulatory approval of the drug substance.

Case study 1: One-step purification using CaptureSelect Factor X Affinity Resin

Factor X is a vitamin K-dependent clotting factor that plays a central role in the coagulation cascade. In its druggable form, it can be used to treat or prevent bleeding in people with hereditary Factor X deficiency.

CaptureSelect™ Factor X Affinity Resin purifies human Factor X from recombinant and plasma sources. Binding is highly selective, with no co-purification of human plasma proteins or host cell proteins, and because the ligand has been developed to bind its target in the presence of calcium, mild elution is possible at neutral pH with EDTA.

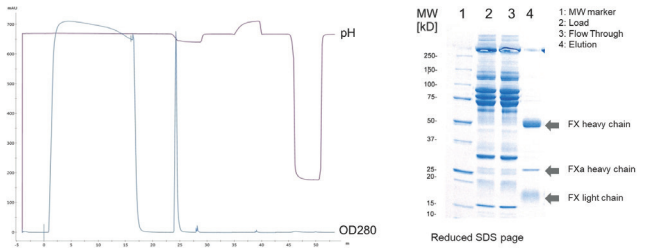

Figure 3. Elution of Factor X from CaptureSelect Factor X Affinity Resin. The chromatogram (left) shows the sharp elution peak resulting from the use of EDTA while the SDS-PAGE gel (right) demonstrates the high purity of Factor X following its extraction from a complex feedstock.

Case study 2: Workflow enhancement with CaptureSelect AAV9 resin

AAV9 is a viral vector that is widely used for delivering gene therapies. A typical AAV9 purification workflow involves three distinct chromatography steps, each of which incurs substantial product losses. These can be condensed into a single chromatography step using CaptureSelect™ AAV9 resin, followed by a polishing step (when needed) which increases yields and shortens timelines (Figure 4).

Case study 3: Baculovirus impurity removal using affinity chromatography

The baculovirus expression vector system (BEVS) is an established platform for producing viral vaccines and gene therapy vectors, benefiting from a robust safety profile, short production times, and high yields. However, because baculovirus is often co-produced with the target, a main challenge of this system lies in separating baculovirus from enveloped virus-like particles (VLPs) or viral vectors.

POROS™ CaptureSelect™ Bacuclear affinity resin was specifically developed for baculovirus impurity removal in vaccine and viral

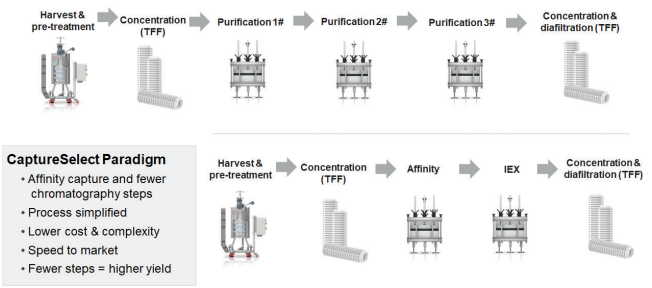

Figure 4. A comparison of non-affinity purification (upper panel) and CaptureSelect affinity purification (lower panel) shows that affinity purification reduces the total number of processing steps required during viral vector purification.

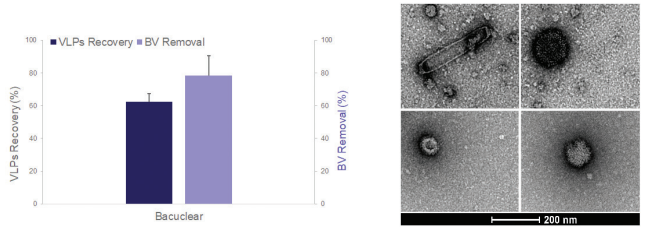

Figure 5. POROS CaptureSelect Baculear affinity resin provides high clearance of baculovirus impurities. An influenza VLP produced in baculovirus demonstrated around 80% baculovirus clearance using the Baculear resin in flow-through mode (left). TEM microscopy images of clarified VLPs (right, upper row) and purified VLPs (lower row) show a significant purity increase.[6]

vector manufacturing. It has been shown to significantly increase the purity of an influenza VLP (Figure 5).

Conclusion

Antibody-based affinity chromatography is a highly versatile tool with broad utility in the production of next generation biologics. As well as reducing the number of process steps, it improves yields and increases purity to deliver a better quality product. In combination, these benefits greatly reduce the time spent on bioprocess development, allowing urgently needed biologic drugs to reach patients sooner.

CaptureSelect™ technology enables one-step purification of many different types of biologic, including newer drug classes. Products for purifying antibody therapeutics, recombinant proteins, and various cell and gene therapies are available for both small-scale studies and process-scale purifications, with further resins and ancillary tools continually being developed.

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CaptureSelect™ chromatography resins: For Research Use or Further Manufacturing. Not for use in diagnostic procedures.