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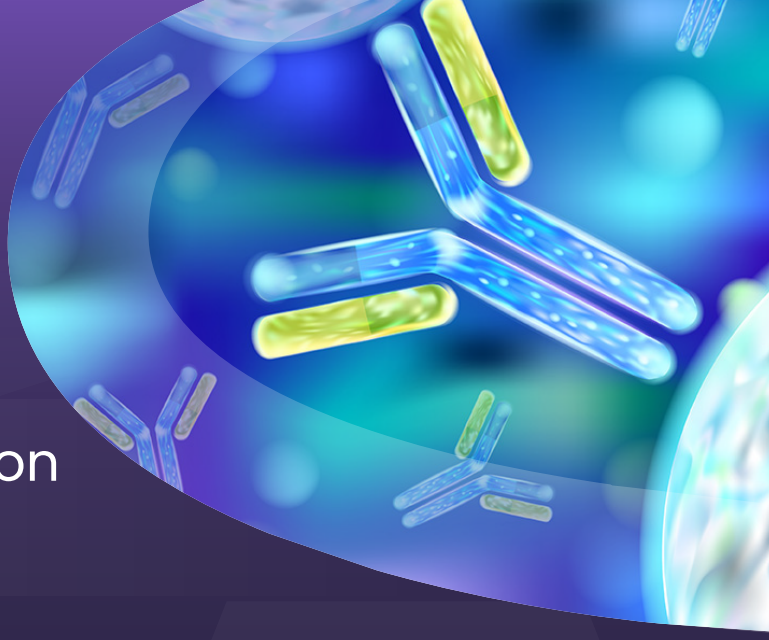
PURITY by DESIGN



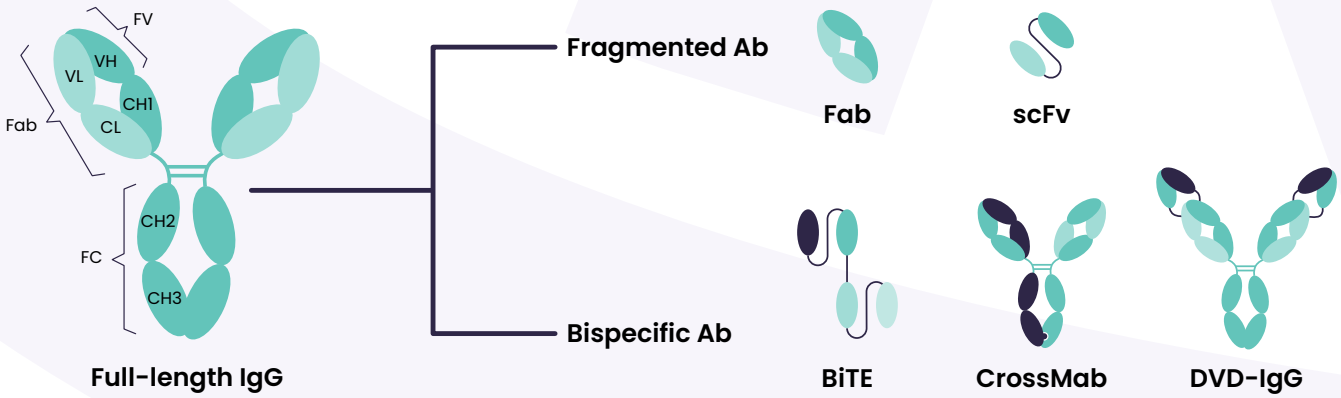
The Discovery and Evolution of Antibody Formats

As antibody therapeutics evolve, new formats such as bispecific antibodies (bsAbs) and Fab fragments offer new and exciting therapeutic opportunities. However, traditional mAb isolation methods are often ineffective, necessitating innovative solutions. This article explores these challenges and introduces Astrea Bioseparations' Fabsorbent™ F1P HF as an alternative strategy for isolating next-generation antibodies.

Antibody-based therapeutics have transformed modern medicine, offering targeted treatments for a range of diseases, including cancer, autoimmune disorders, and infectious diseases. Traditional monoclonal antibodies (mAbs) have been the backbone of this therapeutic class, however, advances in protein engineering have led to the development of various antibody formats, including Fab fragments, single-chain variable fragments (scFvs), and bispecific antibodies. These new formats aim to enhance efficacy, reduce immunogenicity, and improve manufacturability.

Bispecific antibodies (bsAbs) are designed and engineered to recognize and bind two different antigens simultaneously. This dual specificity allows them to "bridge" immune cells interactions to tumor cells and, for example, deliver payloads with high precision. bsAbs have gained significant attention in oncology, particularly for their ability to engage T cells to kill tumor cells. These molecules are also being explored for applications in autoimmune diseases and infectious diseases, where they can modulate immune responses or neutralize multiple viral targets concurrently.

Fab fragments, a type of fragmented antibody, are smaller, antigen-binding antibody fragments consisting of one constant and one variable domain from both heavy and light chains. Unlike full-length mAbs, Fab fragments lack the Fc (fragment crystallizable) region, which reduces their size and allows for improved tissue penetration. This characteristic makes Fab fragments particularly attractive for therapeutic applications where rapid distribution and clearance are desirable.



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In drug discovery, Fab antibodies and bsAbs present several challenges. The absence of an Fc-region in antibody fragments complicate their purification, where traditional methods like Protein A chromatography used for full antibodies, are not applicable. Additionally, the design and production of bsAbs require precise engineering to ensure functional binding to multiple targets, while maintaining stability, yield, and purity. These complexities can increase development timelines, costs, and the need for specialized purification strategies, making the discovery of specific antibody-domain therapies more challenging.

To address these challenges, Astrea Bioseparations has developed a mixed-mode chromatography resin, Fabsorbent™ F1P HF, that provides an alternative purification solution for the effective capture of differing antibody binding regions. Unlike costly Protein A or Protein L strategies, Fabsorbent™ F1P HF uses a synthetic ligand that offers a cost-effective alternative for antibody fragment and bispecific separation.

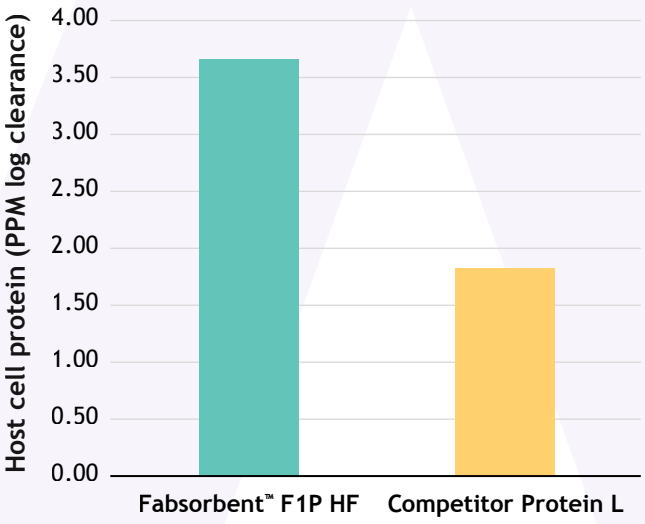
The variable kappa light chain (Vk) domain is one example of the variable antibody region, responsible for antigen recognition. In bsAbs and Fab fragments, Vk light chains contribute to the specific binding of the antibody or antibody fragment for targeted therapeutic applications and serve as a critical determinant of antibody specificity and affinity. In recent years, Vk-containing antibody fragments have been increasingly utilized in therapeutic applications due to their high stability and favorable binding properties. An example of this is a Vk-containing bsAbs designed for cancer immunotherapy, using the Fab Vk-domain to target two distinct tumor antigens while engaging immune effector cells. This approach enhances tumor targeting and reduces off-target side effects, making it a promising candidate for clinical development.

In a case study, a 1 mL high-throughput Fabsorbent™ F1P HF column was compared against a relevant competitor Protein L product for the purification of Vk fragments from a crude *E. coli* feedstock. Purification performance analysis showed comparable elution recovery of Vk fragments between the two adsorbents, with Fabsorbent™ F1P HF demonstrating higher purity. Optimized conditions for Fabsorbent™ F1P HF worked more favorably at pH 9.0 equilibration, followed by an elution step in acetate buffer at pH 5.0. In comparison, the competitor product, post-optimization, utilized wash conditions at pH 3.5, followed by an elution step at pH 2.5. The need to use acidic conditions in downstream processing to obtain optimized product recovery can lead to an increase of protein denaturing and aggregation, which can be exacerbated when scaled up in clinical development.

Reverse-phase HPLC			
Adsorbent	**Sample**	**Amount (mg/mL adsorbent)**	**Recovery (%)**
Fabsorbent™ F1P HF	Load	14.58	N/A
	Elution	11.26	77.28
Competitor Protein L	Load	16.56	N/A
	Elution	12.94	78.14

Table 1: Product recovery comparison using Fabsorbent™ F1P HF and a competitor Protein L using reverse phase HPLC

As the demand for novel antibody formats grows, efficient screening of purification conditions becomes essential. Astrea Bioseparations has a broad range of purification resins, ranging from capture to polish, for mAbs and other antibody formats.



Using Astrea Bioseparations' toolbox approach, products such as Fabsorbent™ F1P HF, HCPure™, Q PuraBead®, and SP PuraBead® Edge provide the market with additional choices for downstream processing workflows. Resins are provided in high-throughput screening formats to streamline discovery and purification. Combining smarter purification strategies and high-throughput screening options will help shape the future of biopharmaceutical development and commercialization of new drugs.

Astrea Bioseparations is a world class provider of chromatography adsorbent and resin services. With over 30 years of chromatography manufacturing expertise, we deliver a unique and trusted service in close partnership with our clients. For more information, please don't hesitate to reach out at sales@astrea-bio.com or visit astreabioseparations.com.