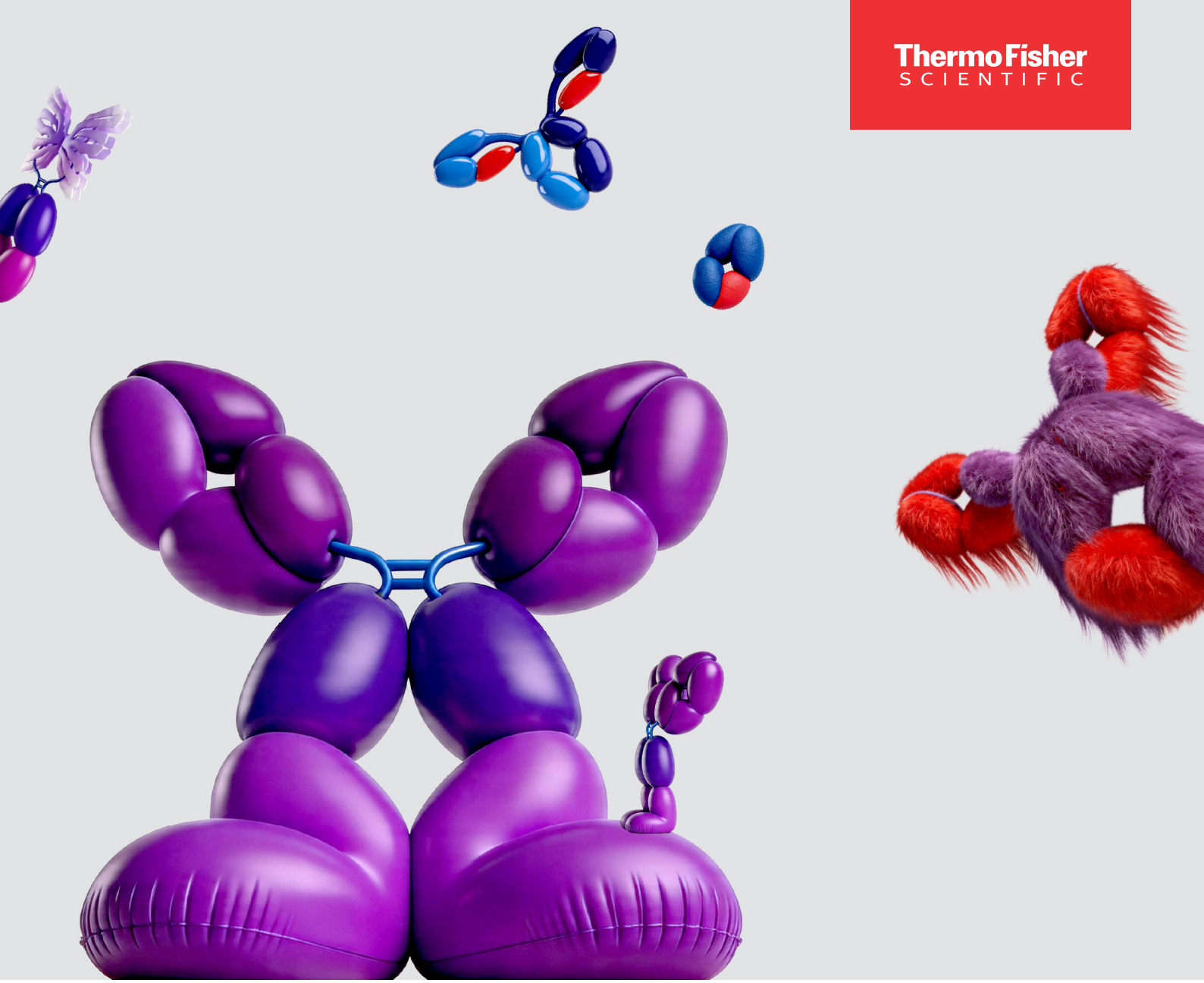

Purification techniques for next-generation antibody therapeutics

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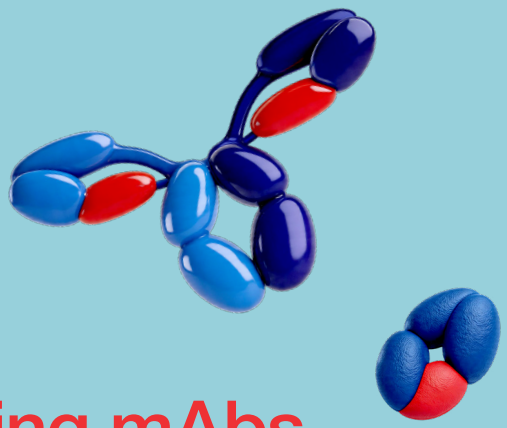

About misbehaving mAbs

Therapeutic antibodies are biopharmaceuticals that are specially designed to bind disease-causing molecules. They help in the treatment of different conditions including cancer, infectious diseases and inflammatory disorders.

Different antibody formats, such as antibody-drug conjugates, bi-specific antibodies and antibody fragments, have been developed in recent years to address different therapeutic needs.

These new formats have significant potential therapeutic value, but they may not always “behave” in the manner of traditional antibodies. Hence, development and manufacturing processes need to be optimized to help maximize efficiency. However, the development of novel antibody modalities presents new purification challenges that traditional affinity resins cannot always solve.

This eBook reviews various therapeutic antibodies modalities available and presents innovative purification tools to address challenges in downstream processing.

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Purification of novel therapeutic antibodies: challenges and opportunities

Introduction

Therapeutic antibodies are biopharmaceutical products designed to bind specific molecules in the body and mimic the adaptive immune response.[1,2,3] They have a wide variety of applications, including the treatment of infectious and inflammatory diseases, as well as certain types of cancer.[4,2,5] While therapeutic antibodies can be complex to produce, they offer a more precise approach to treatment than synthetic small molecule drugs.[6] By engineering antibodies to target specific disease-causing molecules, scientists can direct treatment to affected tissues. This can minimize side effects and helps improve efficacy. As a result, therapeutic antibodies have become a large part of the broader therapeutics market with numerous FDA-approved drugs now available for clinical use.[2]

The basics of antibody structure and function

Naturally occurring antibodies – also known as immunoglobulins – are Y-shaped proteins made up of two identical Heavy chain and two identical Light chain polypeptides (Figure 1).[7,8] The polypeptide chains are arranged into two mirrored heterodimers linked by disulfide bridges.[8,9] Each heterodimer includes one heavy chain and one light chain which are connected by a disulfide bond. Each heavy chain and light chain consist of two regions – one variable (V_H and V_L respectively) and one constant (C_H and C_L respectively). Variable regions are highly specific and define the antigen-binding properties of the antibody, while the constant regions are more conserved.[10] The isotype of an antibody is determined by the constant regions of the heavy chains. There are five isotypes (IgA, IgD, IgE, IgG, or IgM) and all are associated with a different effector mechanism. While IgG is the most extensively used isotype in antibody therapy, other isotypes have also been explored in certain contexts.[11]

The structure of an antibody can be separated into two functionally distinct fragments; the Fc-region and the fragment antibody-binding region (Fab). The Fab region is composed of heavy and light chains and contains the antibody binding sites.[7] Through protein engineering, Fab fragments can be improved for specificity, stability and binding affinity.[12] Hence, Fab fragment design may be carefully considered during the development of novel therapeutics. The fragment crystallizable (Fc) domain is located at the base of the antibody and interacts with effector molecules and cells.[12,13] During an immune response, the Fc region is recognized by complementary receptors on the surface of immune cells. Fc receptor binding triggers various immune functions such as

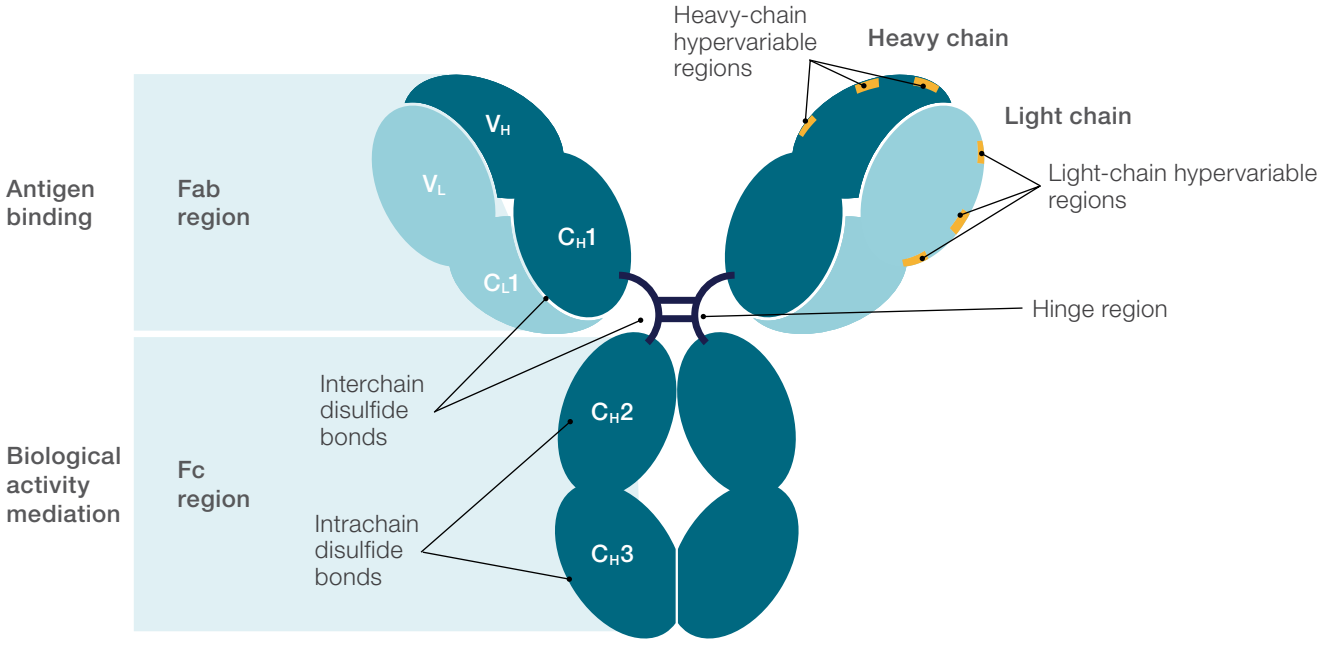

Figure 1: Antibody structure

phagocytosis, antibody-dependent cellular cytotoxicity, and complement activation as well as regulating the half-life of immunoglobulins.[13] Hence, Fc regions can also be engineered to modify the effector functions and stability of therapeutic antibodies.

Therapeutic antibody modalities

Traditionally, the technology used for therapeutic antibodies has centered around engineered monoclonal immunoglobulins.[2] Monoclonal antibodies (mAbs) are generated by protein engineering in mammalian cell lines and are designed to target a single specific antigen.[14] Although mAbs have been successful in treating various diseases, their immunogenicity and limited tissue penetration make them unsuitable for certain therapeutic applications.[15,16] In recent years, the development of multiple, novel antibody modalities has opened up new possibilities for treating a wider range of diseases with greater efficiency (Figure 2).[12]

Antibody-drug conjugates (ADCs) have been developed as a targeted immunotherapy approach that increases the efficacy of mAbs.[17] They combine antibody specificity with the cytotoxic effects of small-molecule drugs to target a specific protein or cell. Hence, ADCs pose a convenient alternative to traditional chemotherapeutic agents, which are both non-specific and toxic to non-cancerous cells.[18] There are currently several FDA-approved ADCs available on the therapeutics market.[17]

Additionally, bispecific monoclonal antibodies (BsAbs) have been designed to address the limitation of single antigen binding.[19] BsAbs can simultaneously bind two targets, such as two distinct antigens or two different epitopes on the same antigen. In structure, they are either IgG-like and contain two heterologous Fc regions, or non-IgG-like and comprise linked variable regions without Fc domains.[20,19]

Some novel therapeutic modalities, such as Fab fragment and Fc-fusion proteins, comprise of partial mAb sequences (Figure 2).[12,21] Fab fragment modalities are smaller in size and may be less costly to manufacture due to their reduced need for glycosylation.[22] They consist of the variable domain and the first constant domain of a mAb HC and LC.[23] Fab fragments offer superior efficacy due to their ability to penetrate hard-to-reach tissues.[24] On the other hand, Fc-fusion proteins consist of an Fc domain fused to a bioactive peptide – such as a cell-specific epitope or receptor.[25] As they don't have Fab regions, Fc-fusion proteins do not have the typical antigen-binding characteristics.

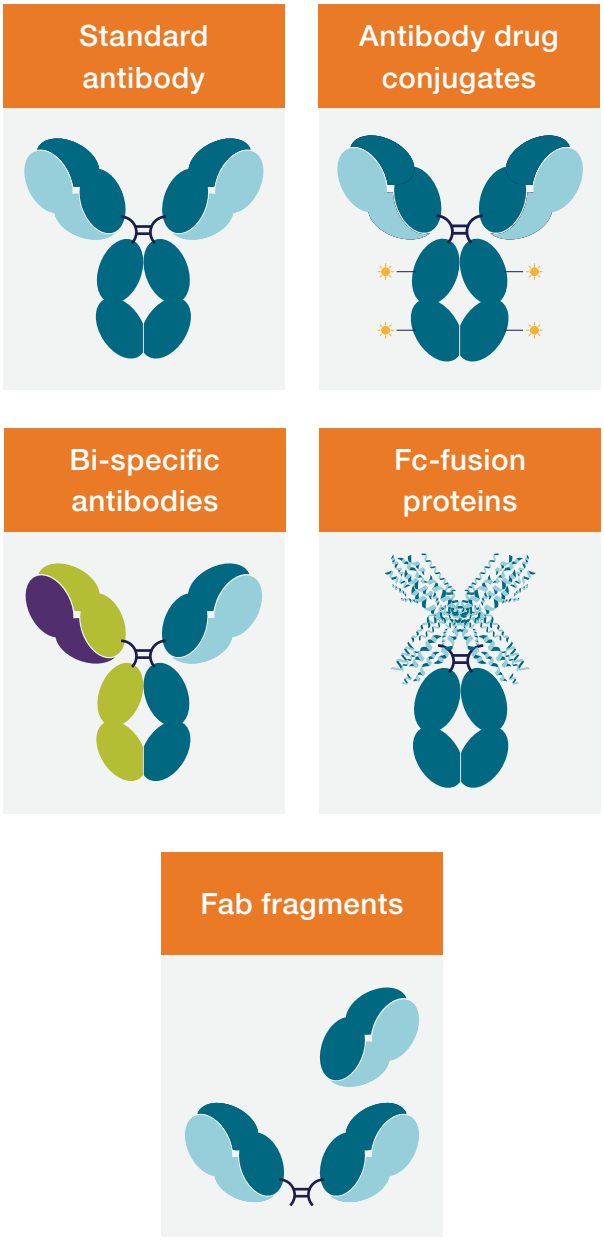

Figure 2. Therapeutic antibody modalities

Producing novel therapeutic antibodies

The bioprocessing of therapeutic antibodies is a complex, multi-step workflow that involves production, purification and concentration (Figure 3).[26,27,28,29] Firstly, raw material selection involves rigorous testing to ensure that product quality and efficiency can be achieved.[30] Upstream processing typically begins with the expression of engineered proteins in mammalian cell cultures.[14] The cells are grown in large bioreactors under carefully controlled conditions optimized for antibody production. Once the cells have produced enough protein, the supernatant is harvested, and the antibodies are purified which can be done through class-specific capture chromatography.[31]

Capture chromatography utilizes resins made from cross-linked matrices and a covalently attached ligands to provide highly specific binding to a target antibody molecule. While different resins can be used, the most commonly used ligand is Protein A. Protein A occurs naturally in some bacteria and has a high affinity for the Fc region of the IgG antibody isotype. Thus, resins containing Protein A capture antibodies while other unwanted proteins and impurities are not retained and are washed away. Captured antibodies can then be eluted under conditions that disrupt the Protein A-Fc region interaction, resulting in highly purified antibodies.

While affinity chromatography is typically used for antibody purification from crude cell culture supernatants, ion exchange (IEX) or hydrophobic interaction chromatography (HIC) is used to remove high- and low-molecular weight aggregates, and process-related impurities during the polishing step.[32] During IEX, antibody fragments are separated based on their net charge and eluted by changing pH or ionic strength conditions. HIC separates proteins based on their hydrophobicity and elutes according to a change in salt concentration.[33] Sometimes, several polishing steps are required in order to achieve sufficient product purity, however, this can result in loss of yield and contribute to overall workflow inefficiency.[32]

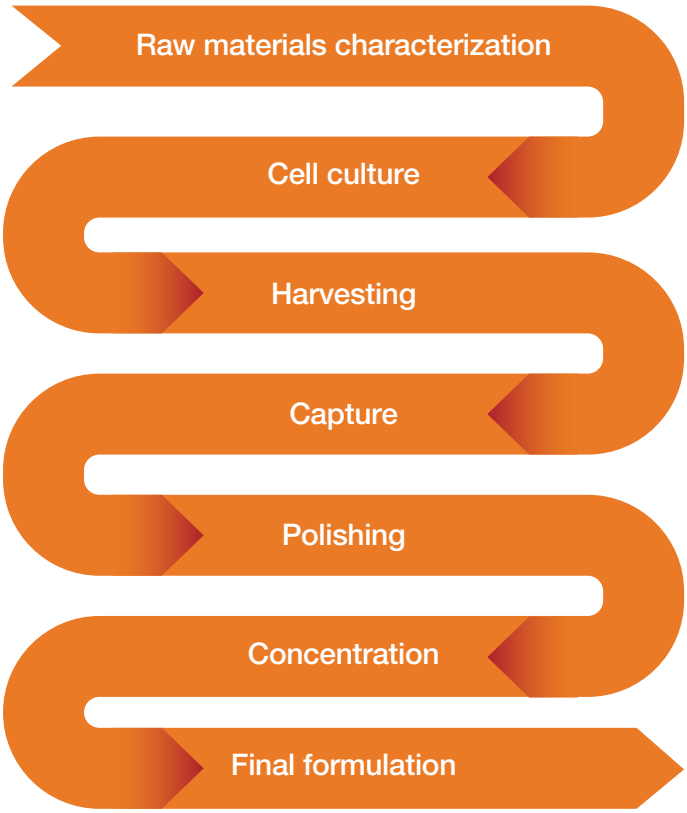

Figure 3: An overview of the antibody bioprocessing workflow

Challenges in therapeutic antibody purification

The increasing complexity and diversity of therapeutic antibodies pose significant challenges to their downstream processing. Different antibody modalities have different physicochemical properties and hence require different purification strategies.[34,35] For example, modalities that lack or have altered Fc regions cannot be purified by Protein A affinity chromatography.[31] Hence, alternative solutions have been developed.

Fab fragments lack the Fc-region and require purification through resins that have been specifically designed for Fab fragment purification. Engineered antibody molecules are often sensitive to lower pH conditions and the likelihood of antibody aggregation is closely linked to the pH of chromatographic eluents. It is therefore essential the right chromatography resins are selected to support gentle antibody purification.

During each step in the antibody purification process, a proportion of the product may be lost or degraded leading to reduced yield and poor workflow efficiency.[32,35] Multiple purification steps may be required to help ensure that quality standards are met, however, each additional step can lead to the potential loss of product and contribute to high workflow costs. Achieving high yield at low cost is essential if antibody bioprocessing workflows are to be platformed, standardized, and clinical demands met. Solutions revolve around the use of affinity chromatography resins specifically developed to bind antibody-subdomain regions. This technology is based on single domain (V_H) antibody fragments immobilized on a chromatography matrix. These resins enable increased purity and yield in a single step and are optimized to operate at higher pH compared to Protein A resins.[35,36]

Moving forward in therapeutic antibodies production

Novel therapeutic antibodies exist in multiple modalities and can be used to treat a variety of diseases.[2,4,5,6] As antibody technologies develop so too do the production and purification requirements for commercial-scale bioprocessing. While challenges remain in the development of standardized platforms, advanced purification solutions for antibody capture and polishing can offer exceptional flexibility and efficiency.[31] Versatile, high-affinity resins can support the platform development of large-scale bioprocessing. By offering superior selectivity across a range of antibody subtypes, such purification solutions support scientists in the rapid development of a wide variety of therapeutic applications. Additionally, improved purification tools enable the development of safer, more effective, antibody solutions that align with rigorous regulatory standards.

Overall, efficient and effective purification of antibodies can support advanced antibody-based therapeutics.[34,35]

Learn more about our latest antibody purification solutions available

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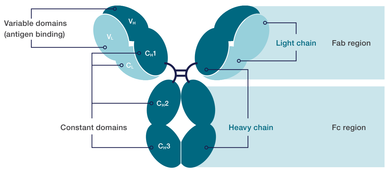
Purification solutions for novel antibody modalities

Therapeutic monoclonal antibodies (mAbs) are customized biopharmaceuticals used for the diagnosis and treatment of various diseases. Traditionally, Protein A resins have been considered the workhorse for the purification of mAbs. However, the growing diversity of therapeutic antibody modalities in the drug pipeline has revealed a need for new purification tools. This infographic explores affinity resins specifically designed for the purification of different types of antibody modalities.

Antibody structure

Since the 1980's numerous mAbs have been approved for therapeutic use. Based on the IgG antibody structure, a ~150 kD protein structure comprised of two identical light chains and heavy chains. Together these chains form a flexible Y-shaped structure.



IgG antibody

Antibodies can be engineered to create novel therapeutic variants. mAbs are homogenous preparations of antibodies, making them identical in protein sequence, antigen-binding recognition, affinity and downstream functional effects.

Therapeutic antibody modalities



Standard monoclonal antibody

The first antibody modality to be used as a therapeutic agent. It has the basic IgG structure and binds to a unique epitope of an antigen to activate or inhibit biological processes that are implicated in a specific disease.



Antibody-drug conjugate (ADC)

mAb chemically linked to a drug or toxin. ADCs are used to deliver the drug to a specific target protein on a cell. This therapeutic modality is often used to attack cancer cells.



Bi-specific antibody

Engineered mAbs that can simultaneously bind to two different antigens or two epitopes on the same antigen. The dual functionality opens up a wide range of applications used in a variety of disease pathways.



Fc-fusion proteins

Fc domain of a mAb is fused to a functional protein. Fc-fusion proteins are often created to prolong half-life of the therapeutic protein. Fc-fusion proteins do not have antigen-binding properties like mAbs have.



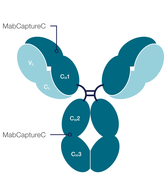
F(ab) and F(ab')2 fragments

Consist of the variable domain and the first constant domain of each heavy and light chain. These small molecules can enhance pharmacokinetic properties and increase efficiency of tissue or tumor penetration.

Purification of antibody therapeutics

Therapeutic mAbs need to be produced at large scale. Over time, production processes, upstream as well as downstream, have been optimized to deliver high yields and to maximize efficiency.

Traditionally, Protein A affinity chromatography has been the method of choice for the purification of mAbs.



How do Protein A resins work?

Native Protein A is a 55-kDa bacterial cell wall protein that naturally binds to IgG molecules. Because of these binding properties, Protein A can be used as a ligand on a chromatography support and used for the purification of mAbs.

The Thermo Scientific™ MabCaptureC Affinity Matrix is a high-performance Protein A resin specifically designed to improve the purification efficiency of mAbs with intact Protein A binding sites.

Purification challenges of novel antibody modalities

The development of novel antibody modalities introduced some challenges in the purification process meaning Protein A affinity resins are not always effective.

Absent or altered Protein A binding site



pH sensitive molecules (e.g., bi-specific Ab, Fc-fusion proteins)



Formation of dimers and/or aggregation (e.g., bi-specific Ab)



Elevated levels of high and low molecular weight species



Overexpression of light chains and light chain dimers



Antibody-subdomain specific purification

Affinity chromatography resins specifically developed to bind antibody-subdomain regions can be the solution to these challenges. Thermo Scientific™ CaptureSelect™ technology is based on single domain (V_HH) antibody fragments immobilized on a chromatography matrix.

CaptureSelect antibody affinity resins enable increased purity and yield in a single step and are optimized to operate at higher pH compared to Protein A resins.

Thermo Scientific™ CaptureSelect™ CH1-XL Affinity Matrix



- Affinity for the C_H1 domain
- Purification of all mAbs and Fabs irrespective of light chains
- Does not bind free light chains (often product-related impurities)

Thermo Scientific™ CaptureSelect™ KappaXP Affinity Matrix CaptureSelect™ LambdaXP Affinity Matrix



- Affinity for C_L-Kappa or C_L-Lambda domains
- Purification of Fab fragments and bi-specific Abs
- High dynamic binding capacity
- Mild elution

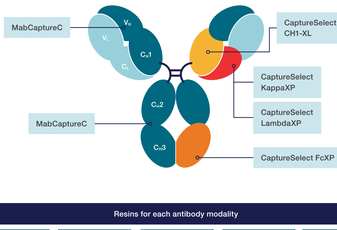
Thermo Scientific™ CaptureSelect™ FcXP Affinity Matrix



- Affinity for the C_H3 domain
- Purification of mAbs with altered Protein A binding site and Fc-fusion proteins
- Mild elution

For research use or further manufacturing. Not for diagnostic use or direct administration into humans or animals.

Find the purification solution that best fits your needs



Resins for each antibody modality

Standard antibody	Antibody-drug conjugates	Bi-specific antibodies	Fc-fusion proteins	Fab fragments
				
MabCaptureC FcXP	MabCaptureC FcXP	FcXP CH1-XL KappaXP LambdaXP MabCaptureC	FcXP MabCaptureC	CH1-XL KappaXP LambdaXP

Discover more at
thermofisher.com/antibody-derived-therapeutics

Polishing pathways: Enhancing antibody purity and performance



Monoclonal antibodies (mAbs) are transforming medicine, redefining standards in oncology, immunology, and beyond. However, the rapid advancement of mAb therapeutics is now outpacing the capacity of downstream bioproduction workflows. Modern formats such as bispecific antibodies (BsAbs) and Fc-fusion proteins, as well as increased production levels of traditional mAbs, are creating new challenges in purification.[1]

This infographic provides a simple guide for selecting the appropriate polishing chromatography methods to address impurity removal challenges at different scales.

Core challenges in modern mAb purification

High titers of standard mAbs



With improved upstream efficiency, more product can mean more impurities.[2]

Misbehaving mAbs



Some antibodies are tricky to purify, as they can misfold, mispair, and aggregate.[3]

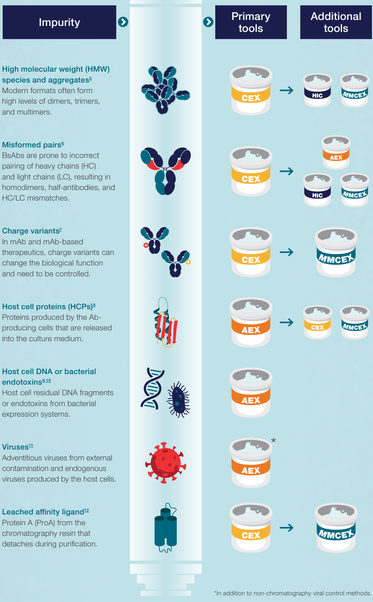
Modern formats



Advanced mAb formats bring additional complexity, often requiring tailored purification strategies.[4]

Matching impurities with the right solution

Different mAb structures, formats, and process conditions are prone to different types of impurities. To support the purity and stability of the final product, these impurities must be removed efficiently—often requiring a combination of chromatography modalities including anion exchange (AEX), cation exchange (CEX), hydrophobic interaction (HIC), mixed-mode (MMC) chromatography, and mixed-mode cation exchange (MMCEX).



*In addition to non-chromatography viral control methods.

Overcoming impurities from bench to manufacturing scale

Powered by Thermo Scientific™ POROS™ technology, our vast portfolio of polishing resins offers the tools for the consistent and reliable separation of impurities from small- to large-scale manufacturing.



Not sure which Thermo Scientific™ POROS™ resin is right for your purification process?

Check out the Resin Selection Guide

Evaluating resins? Explore our solutions

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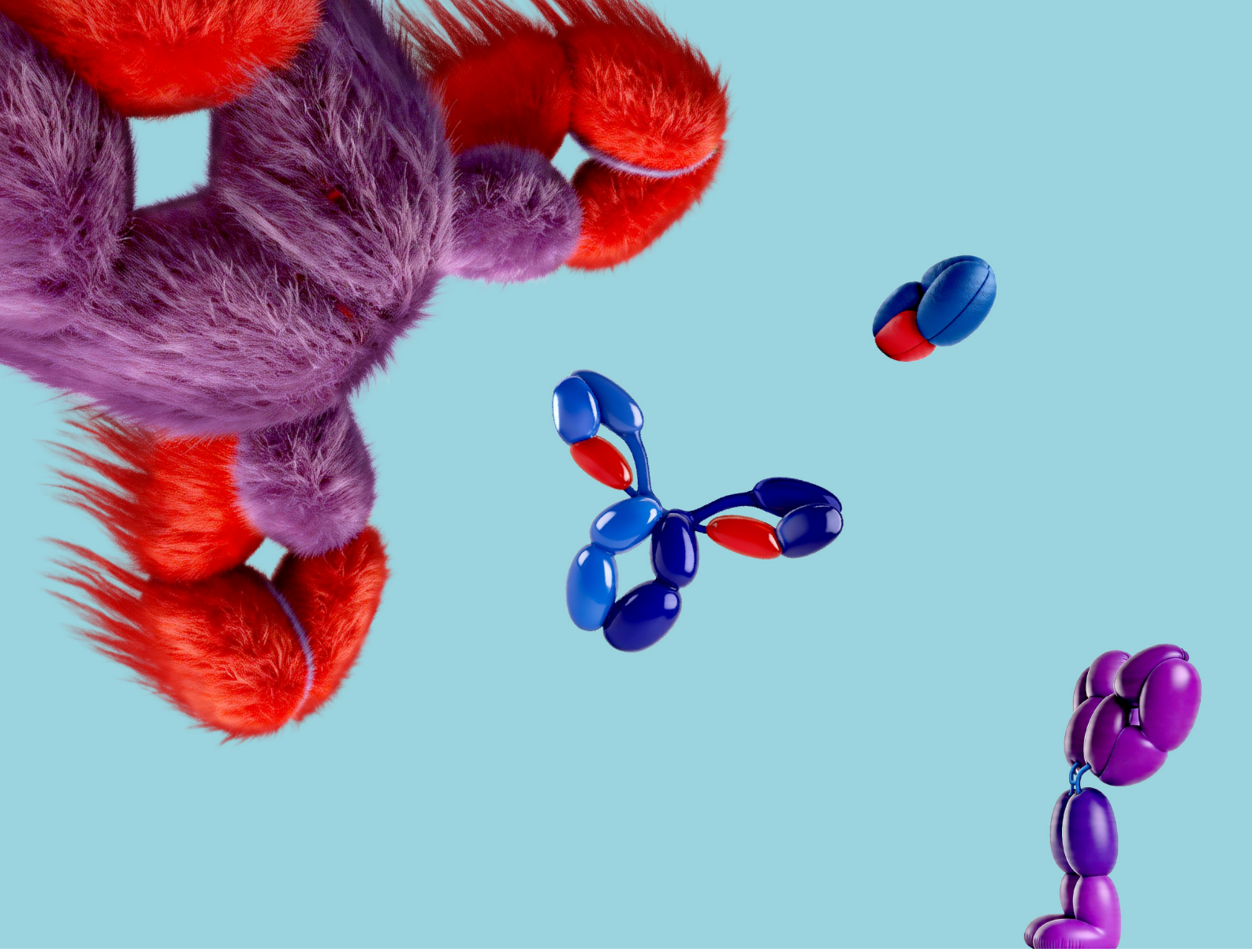

Tools for efficient downstream processing of antibody-based therapeutics

This webinar focuses on tangible chromatography tools designed for specific purification challenges related to mAb purification and overall downstream process success.

Join the conversation and learn how to utilize the chromatography toolbox to improve outcomes in the purification of therapeutic monoclonal antibodies and antibody-derivatives.

Watch now

Innovative capture purification solutions for therapeutic antibody manufacturing

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Thermo Fisher Scientific, Leiden, the Netherlands

Introduction

For decades, affinity purification platforms such as Protein A and Protein L have been crucial in the manufacturing processes of therapeutic monoclonal antibodies. However, as engineered modalities such as bispecific antibodies, fragments, and Fc-fusion proteins emerge, new challenges arise in the downstream processing of these complex molecules. To address these challenges, affinity chromatography resins designed to target specific antibody subdomains offer a promising alternative for purifying these novel formats. This advancement is crucial in enhancing the commercial manufacturing of next-generation antibody therapeutics.

A unique antibody affinity purification portfolio

Supporting manufacturers in the purification of novel antibody formats

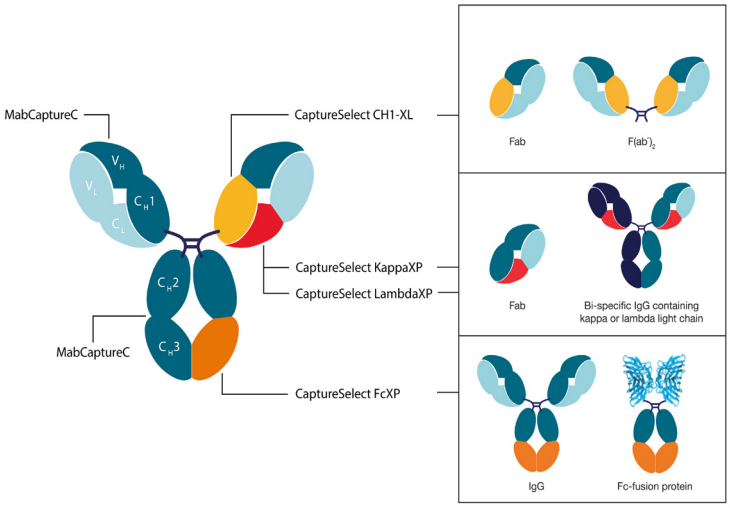


Fig.1. Affinity capture resin selectivity. Infographic showing the binding regions of the Thermo Scientific™ MabCaptureC affinity matrix (Protein A) and all Thermo Scientific™ CaptureSelect antibody affinity resins.

MabCaptureC Affinity Matrix and CaptureSelect™ FcXP Affinity Matrix

The complete platform for IgG-based molecules and Fc-fusion proteins

The **MabCaptureC affinity resin** is a high-performance Protein A resin specifically designed to improve the purification efficiency of mAbs with intact Protein A binding sites. For antibody molecules that lack or have an altered Protein A binding site, or for Fc-fusion proteins, the **CaptureSelect FcXP CH3 subdomain-specific resin** is an ideal solution.

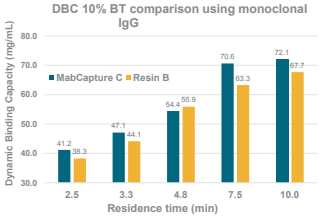


Fig.2A MabCaptureC Resin Dynamic Binding Capacity comparison at increasing residence times. The resin was compared to commercially available, alternative Protein A resin (Resin B).

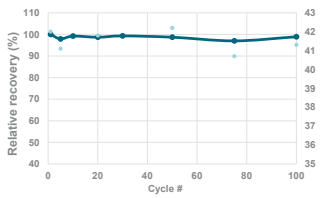


Fig.2B MabCaptureC Resin Reusability study. The resin shows excellent alkaline stability. No decline is observed after cleaning with 0.2M NaOH over 100 cycles. CHO expressed **Rituximab**.

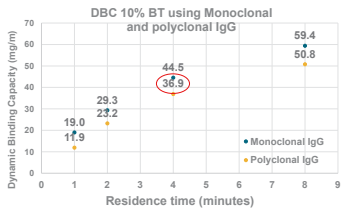
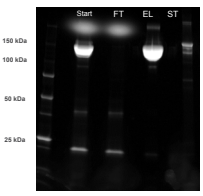


Fig.3A CaptureSelect FcXP Resin Dynamic Binding Capacity. The resin shows a high DBC; > 40 g/L (10% BT/ 5 min residence time) - **Rituximab**.



- Start - **Rituximab**, monoclonal human/mouse chimeric IgG1 (1.7 g/l)
- FT – flowthrough fraction
- EL – 10 x diluted pH 4.0 elution fraction,
- ST – pH 2.0 strip

Fig 3B. One-step purification from crude material with high purity. Overexpressed light chain dimers are present in the flow through (FT) but not in the elution fraction (E). ST = strip pH 2

Thermo Scientific™ CaptureSelect™ CH1-XL Affinity Matrix

A scalable platform solution designed to purify Fab-fragments

- Binds to the CH1 domain
- No co-purification of free light chains (only correctly assembled Fabs)
- Efficient elution at milder pH (4 – 4,5)

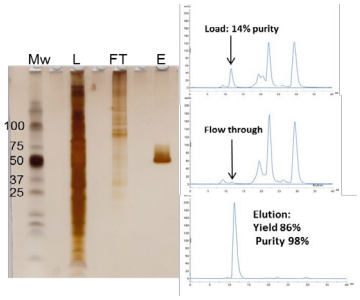


Fig. 4. Ranibizumab feed from HEK293 cells (Fab fragment). Purification shows high yield and purity in a single step

Left: SDS-PAGE silver staining of the load (L), flow-through (FT) and elution (E) fractions, showing no presence of light chains in the elution pool.

Right: Gel filtration analysis showing 98% purity of the Fab fragment in the elution fraction with a yield of 86%

CaptureSelect™ KappaXP Affinity Matrix & CaptureSelect™ LambdaXP Affinity Matrix

Solving purification challenges for bi-specific formats

- Binds to constant domain of Kappa or Lambda light chain
- High dynamic binding capacity (tested with Bi-specific mAbs)
- Efficient elution at milder pH

Thermo Scientific resin	Dynamic Binding Capacity (human IgG)	Elution properties
CaptureSelect KappaXP resin	40 g/L at 2 min residence time	Efficient elution at milder conditions (pH 5-6) with additives
CaptureSelect LambdaXP resin	> 35 g/L at 4 min residence time	Efficient elution at pH 3.5-4 – small elution pool volume

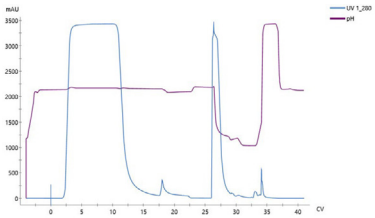


Fig.5. Elution performance of CaptureSelect LambdaXP resin. Efficient elution (3 CV) of a bi-specific antibody at pH 3.6 using a load concentration of 32 mg/mL

In summary

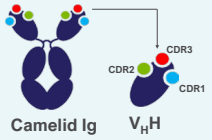
The Thermo Scientific™ antibody purification toolbox enables process developers to address the challenges that arise during the production of new therapeutic antibody modalities.

This portfolio of resins helps to:

+ **Obtain high purity and yields in a single capture step**
+ **Reduce process steps**
+ **Easily upscale for larger manufacturing batches**

CaptureSelect Technology

- Technology based on single-domain antibody fragments [V_HH]
- High target purity in a single step, independent of feedstock
- Innovative screening technology to determine final resin properties:
  - target specificity
  - mild pH elution
  - ligand stability
- Scalable & animal origin free technology
- Suitable for cGMP manufacturing processes



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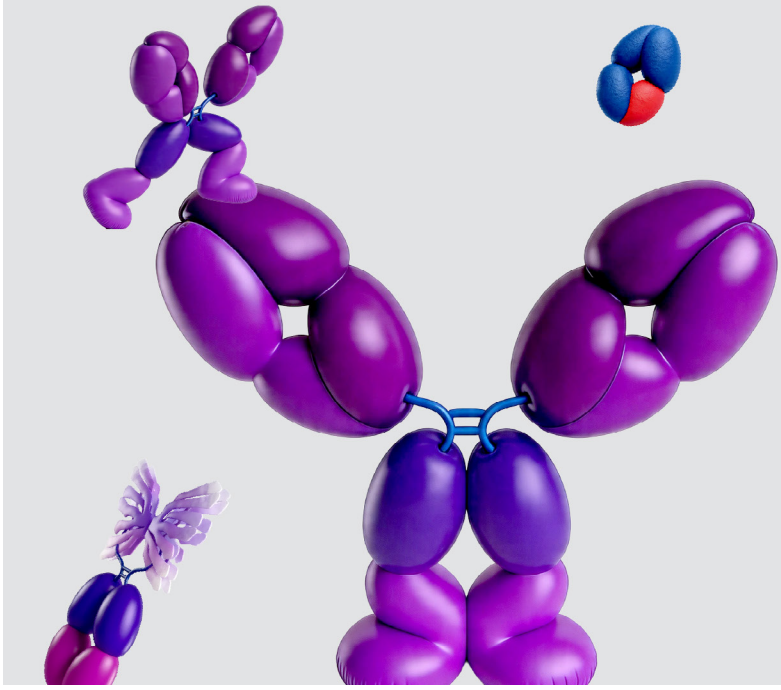
Learn more at **thermofisher.com/antibodypurification**

ThermoFisher SCIENTIFIC

Resources



Learn about MabCaptureC, a high-performance Protein A chromatography resin, specifically designed to enhance the efficiency of antibody purification workflows.

Read more



Gain insights into the different affinity chromatography resins designed to solve the purification challenges of next-generation antibody therapeutics.

Read more

Learn more at **thermofisher.com/antibody-therapeutics**

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