Application Case 2:

In this case, affinity chromatography and ion-exchange chromatography were employed for the purification of IgG-like bispecific antibodies (BsAbs).

The process route: Protein SupAt Beads → Smac SP40 → Smac Q40

For polishing, Smac SP40 was preferred to remove aggregates and host cell proteins (HCP). Throughout the purification process, the quality of the stoste fully met the established quality requirements (Fig. 7–8 & Table. 2).

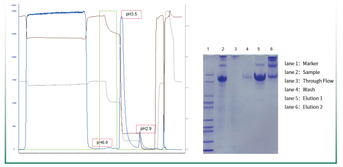

Fig 7.Protein SupAt Beads chromatography and electrophoresis.

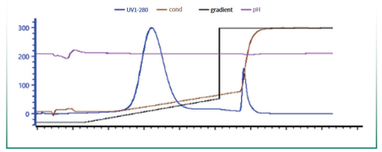

Fig 8. Smac SP 40 chromatography.

Table 2. Chromatography Results.

Step	Mode	Purity (%)	Aggregates (%)	Fragment (%)	HCP (ng/mg)	HCD (pg/mg)	Ligand Residue (PPM)
Protein SupAt Beads	Binding	93.62	5.42	0.83	347.9	376	3
Smac SP 40	Binding	98.55	0.46	0.74	15.1	336	0
Smac Q 40	Binding	99.53	0.09	0.36	7.6	0	0

Non-IgG-like BsAbs: ▸▸▸

For non-IgG-like bispecific antibodies, it is essential to consider the type and structure of impurities, as well as their differences from the target molecule, when selecting a purification method. Choosing the appropriate affinity resin based on the specific domain can significantly enhance purification efficiency.

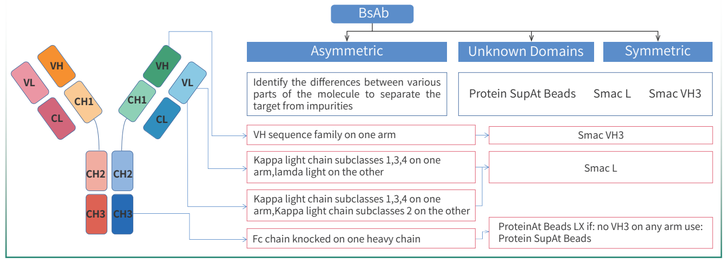

Fig 9. BsAb' s structure has a big influence on the selection of affinity chromatography.

The ligand of Smac L is an alkaline-resistant recombinant Protein L, which specifically binds to the κ light chain of antibodies and has a broader binding range for various immunoglobulin classes and subclasses. Cleaning-in-place (CIP) can be performed using a NaOH solution.

Antibody heavy chain variable domains(VHH/nanobody):

VHHs have partial or complete functions of full antibodies, with a smaller molecular weight, offering a wide range of applications and promising development prospects.

In the purification process, VHHs are more diverse than mAbs and can be purified by Smac VH3, along with ion-exchange chromatography, hydrophobic chromatography, and multimodal chromatography.

The fusion protein strategy provides a specific binding site. After enzymatic hydrolysis to remove the tag, high-purity antibody stoste can be obtained through 1-2 purification steps (Fig. 10).

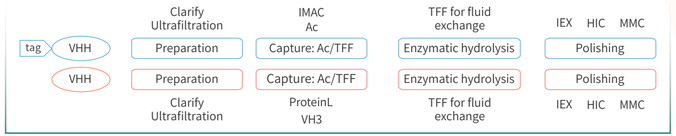

Fig 10.Two purification protocols for VHH.

Smart-Lifesciences provides a comprehensive range of solutions for antibody drug development, offering support services such as process development and personnel training at each stage of development and production. Our product offerings include small-scale prepacked columns for R&D, as well as chromatographic resins produced at kiloliter scale. We ensure completely independent development and production, achieving a stable supply.

Product Advantages: ▸▸▸

1. **High dynamic binding capacity (DBC)**
2. **Long lifespan**
3. **Alkali-resistant and easy to clean**
4. **Low nonspecific adsorption**
5. **High flow rate**
6. **Value-added services**

Product Information

Product	Cat.No.	Size
Protein At Beads LX	SA085	(5ml/25ml/100ml/500ml/1L/10L)
Protein SupAt Beads	SA095	(5ml/25ml/100ml/500ml/1L)
rProtein A-ME Beads 4FF	SA073	(5ml/25ml/100ml/500ml/1L)
Smac L	SA100	(5ml/25ml/100ml/500ml/1L)
Smac MMA	S1031	(25ml//100ml/500ml/1L/10L)
Smac MMC	S1033	(25ml/100ml/500ml/1L/10L)
Smac Q	S1019	(25ml/100ml/500ml/1L/10L)
Smac Q 40	SI035	(25ml/100ml/500ml/1L/10L)
Smac SP	S1027	(25ml/100ml/500ml/1L/10L)
Smac SP 40	SI028	(25ml/100ml/500ml/1L/10L)
Q Beads HP	SI010	(50ml/75ml/250ml/1L/10L)
SP Beads HP	SI011	(50ml/75ml/250ml/1L/10L)

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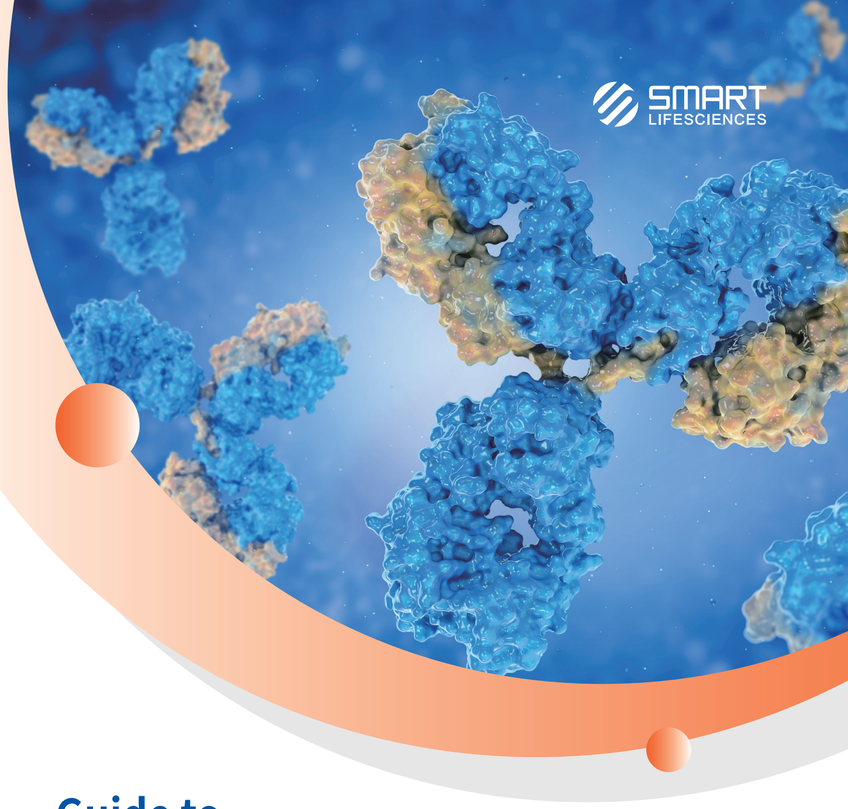

SMART LIFESCIENCES

Guide to Antibody Purification

Professional Manufacturer of Chromatography Resins

Introduction

After decades of development, antibody drugs have become a hotspot in global new drug discovery. Through fragment recombination, drug coupling, site modification, and other methods, researchers have developed monoclonal antibodies (mAbs), bispecific antibodies (BsAbs), polybispecific antibodies (PsAbs), antibody-drug conjugates (ADCs), and antibody fusion proteins, among other antibody drugs.

Monoclonal Antibodies :

Generally, mAb purification involves three chromatography steps: protein A, cation exchange, and anion exchange chromatography, to obtain a qualified drug substance (Fig. 1).

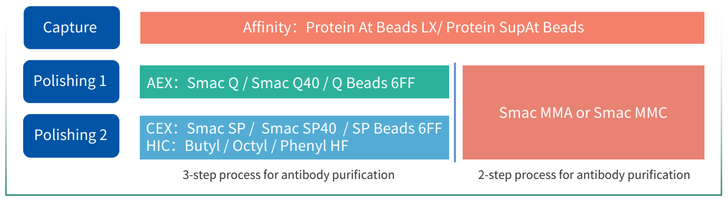

Fig. 1 Three-Step and Two-Step Antibody Purification Protocols for mAbs.

In this process, alkali-resistant protein A affinity chromatography is usually used for the initial capture step.

Affinity chromatography serves as the core step in antibody purification, achieving a purity level exceeding 90% after this stage. Residual impurities, such as host cell proteins (HCP), host cell DNA (HCD), endotoxins, viruses, polymers, and shed ligands, are removed using cation and anion exchange chromatography, or multimode chromatography, to meet final product quality requirements.

Smart-Lifesciences offers high-affinity, alkali-resistant chromatography media, including Protein At Beads LX and Protein SupAt Beads. The company has established a quality control system to ensure texceptional batch-to-batch stability, supporting continuous, stable, and rapid delivery of excellent antibody purification products.

Product parameters:

Protein SupAt Beads undergo rigorous testing to ensure their performance meets design standards (Fig. 2 & 3).

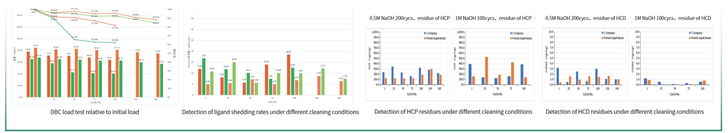

Fig 2. Test data of Protein SupAt Beads.

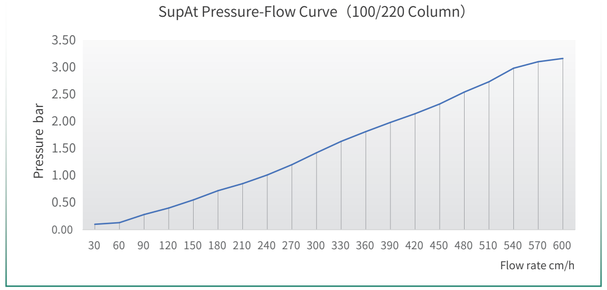

Fig 3. Pressure-flow curve of Protein SupAt Beads.

Protein At Beads LX and Protein SupAt Beads are excellent affinity chromatography resins that have demonstrated good purification performance in several cases.

Application Case 1:

In this case, the monoclonal antibody was purified using a classical 3-step chromatographic process.
The process route: ProteinAt Beads LX → Smac Q → Smac SP40, achieving a total recovery rate of 83%. The impurity removal was effective, with purity approaching 100% (Table. 1 & Fig 4).

Table 1. Chromatography Results.

Step	Mode	Purity (%)	Aggregates (%)	HCP (ng/mg)	HCD (pg/mg)	Ligand Residue (PPM)
Protein At Besds LX	Binding	98.6	0.87	246.3	437	2
Smac Q	Flow Through	99.16	0.3	98.6	0	0
Smac SP 40	Binding	~100	0	8.3	0	0

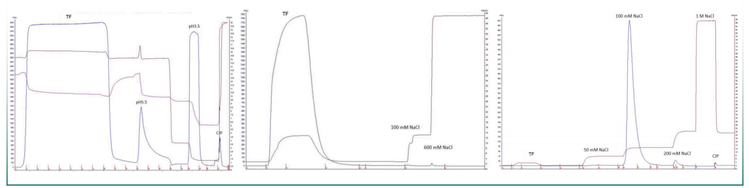

Fig 4. Chromatographic profiles of Protein At Beads LX (left), Smac Q 40 (middle), and Smac SP 40 (right).

Bispecific Antibodies (BsAb) :

The preparation of bispecific antibodies is similar to that of monoclonal antibodies, but it is more complex and challenging. Effectively removing DBPs, such as type dimers, hemi-antibodies, light/heavy chain-related impurities, and polymers, poses significant challenges. The Design of Experiments (DOE) method is recommended to optimize chromatographic process parameters.

The bispecific antibodies can be broadly classified into IgG-like and non-IgG-like structures, based on the presence or absence of an Fc fragment. Additionally, they can be categorized as symmetric or asymmetric structures (Fig. 5).

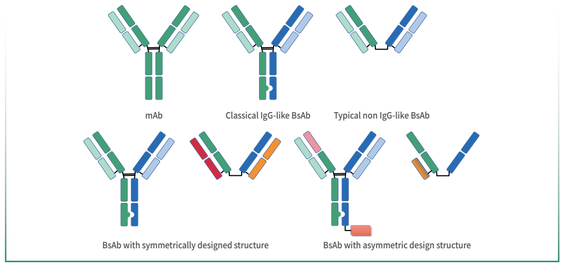

Fig 5. Structural differences between typical mAbs and BsAbs.

IgG-like BsAbs:

In the capture phase, Protein SupAt Beads can be used to bind bispecific antibodies (BsAbs), allowing for the removal of some oligomers and mismatched molecules based on differences in binding ability. For polishing purification, ion-exchange, hydrophobic, or multimodal resins are preferred (Fig. 6).

Smart-Lifesciences provides series of antibody purification resins to adapt to the purification of different bispecific antibodies.

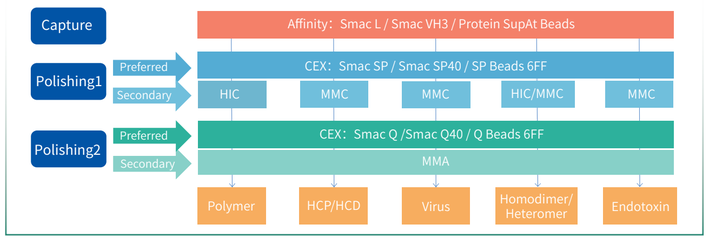

Fig 6. Bispecific antibody purification protocol.