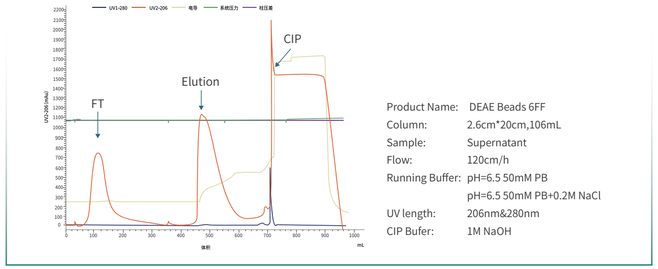
Downstream purification process of pneumonia vaccine



Product Name: DEAE Beads 6FF
Column: 2.6cm*20cm,106mL
Sample: Supernatant
Flow: 120cm/h
Running Buffer: pH=6.5 50mM PB
pH=6.5 50mM PB+0.2M NaCl
UV length: 206nm&280nm
CIP Bufer: 1M NaOH

Figure 6. 5 Chromatographic curve of type 5 pneumococcus

Table 3. Analysis Results

Sample	HCP Occupancyrate(%)	Yield(%)	HCP RemovalRate(%)	HCD RemovalRate(%)
DEAE-Loading	8.35	/	54.34	81.28
FT-1	2.25	2.70		
FT-2	12.36	19.10		
Elution	5.44	70.17		

The result shows that the protein is easier to bind resins in DEAE chromatography, with most HCP appearing in the FT and CIP fractions, while the target polysaccharide is eluted. The protein content in the elution is 5.44%, which is below the 7.5% limit set by the European Pharmacopoeia. In terms of impurity removal, one-step chromatography effectively removes 54.34% of the protein and 81.28% of the nucleic acids, significantly enhancing the safety of the product.

2) However, the purity of tetanus toxoid obtained through traditional methods is often insufficient, adversely affecting its immunogenicity and safety. Currently, column chromatography is widely used in the purification of biotechnological products, employing various chromatographic strategies for antigen purification. Smart-Lifesciences can provide a solution for refining tetanus toxoid, utilizing IEX and HIC two-step chromatography to improve the purity of the target.

Purification Process of Tetanus Toxin Downstream

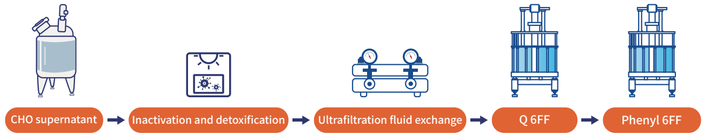
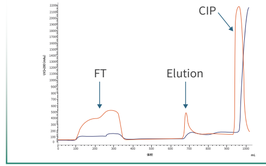
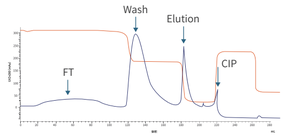


Figure 7. Process route of tetanus purification



Product Name: Q Beads 6FF
Column: 2.6cm*22cm,117mL
Sample: Supernatant
Flow: 120cm/h
Running Buffer: pH=7.6 50mM PB
pH=6.5 50mM PB+0.2M NaCl
UV length: 280nm
CIP Buffer: 1M NaOH

Figure 8.IEX-Q chromatographic curve.



Product Name: Phenyl Beads 6FF(High Sub)
Column: 2.6cm*25cm,132mL
Sample: Supernatant
Flow: 120cm/h
Running Buffer: pH=7.6 20mM PB+1M ammonium sulfate
pH=7.6 20mM PB+0.5M ammonium sulfate
pH=7.6 20mM PB
UV length: 280nm
CIP Buffer: 1M NaOH

Figure 9.HIC-phenyl chromatographic curve.

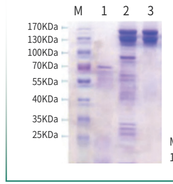


Figure 10.SDS-PAGE

Table 4. Analysis Results

Sample	V (ml)	A280 (mg/ml)	Titer (Lf/ml)	Protrin Yield (Lf/ml)	Titer Yield
Q-Loading	863	3.088	1200	/	52.64%
Q-E	380	1.473	/	21.00%	
HIC-E	188	0.60	2900	20.15%	

After the two-step purification process, the purity of tetanus toxin was significantly improved, as confirmed by both SDS-PAGE analysis and the flocculation test. No obvious miscellaneous bands were observed on the SDS-PAGE, and the results of the flocculation reaction met the requirements outlined in the pharmacopoeia.

Product Information

Product	Cat.No.	Size
Q Beads 6FF	SI001	(25ml/100ml/500ml/1L/10L)
Smac Q 40	SI035	(25ml/100ml/500ml/1L/10L)
Phenyl Beads 6FF (High Sub)	SH006	(25ml/100ml/500ml/1L)
Smac Core 700	SEC028	(25ml/100ml/500ml/1L)
DEAE Beads 6FF	SI005	(25m//100m/500ml/1L/10L)

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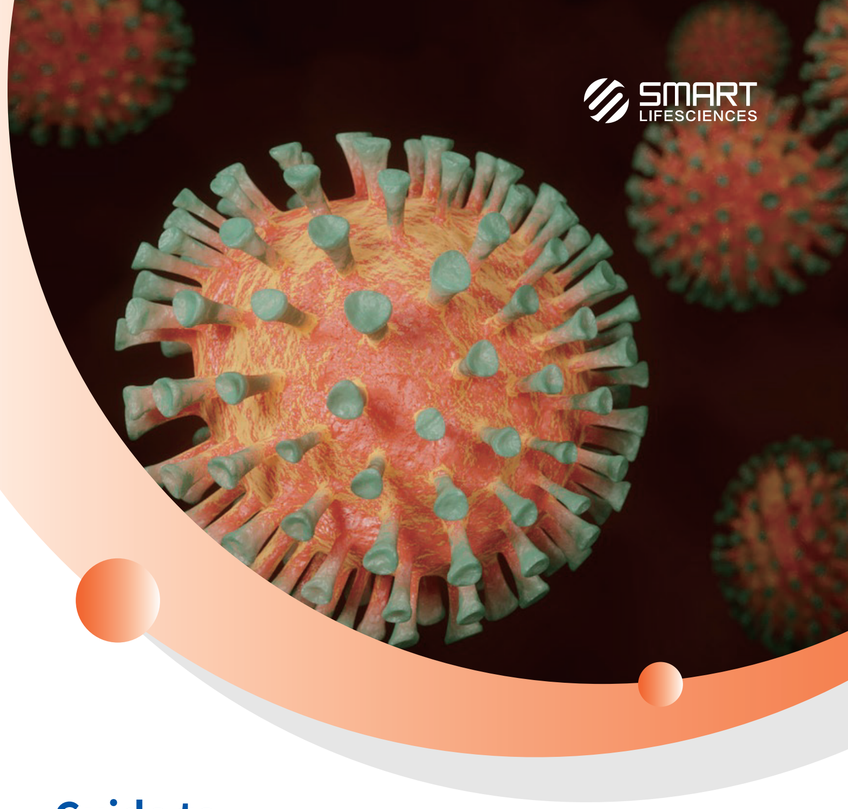


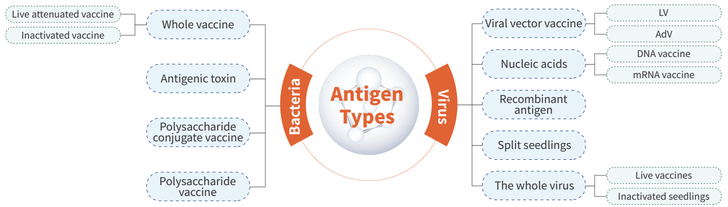
SMART LIFESCIENCES

Guide to Vaccine Purification

Professional Manufacturer of Chromatography Resins

Abstract:

The development of vaccines has undergone multiple technological revolutions, resulting in a wide variety of vaccine types. Different strategies are often used to obtain antigens, such as viral inactivation, nucleic acid delivery, or recombinant protein and polysaccharide conjugated vaccines. Smart-Lifesciences provides a variety of solutions for the recombinant protein vaccines, mRNA vaccines, DNA vaccines, viral vector vaccines and other new vaccines.



Vaccines - Viral Antigens

Vaccines have various forms, with recombinant protein and whole-virus vaccines being the most common. According to statistics, these two types account for approximately 45% of all vaccine categories.

1) Recombinant protein is a kind of antigen vaccine formed by allogeneic expression and purification. Usually, the recombinant antigen contains substantial impurities, that need to be removed through chromatography. Column chromatography, a gentle purification method that effectively increases purity, is the preferred strategy for most commercial vaccines. Smart-Lifesciences offers flexible solutions for recombinant protein vaccine cases. The RSV purification process route is shown in the diagram below:

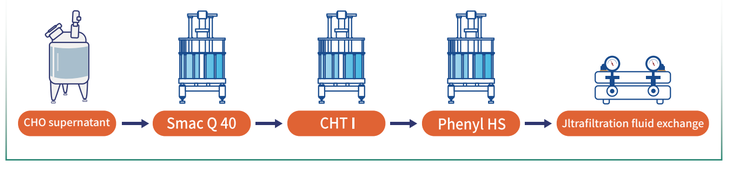
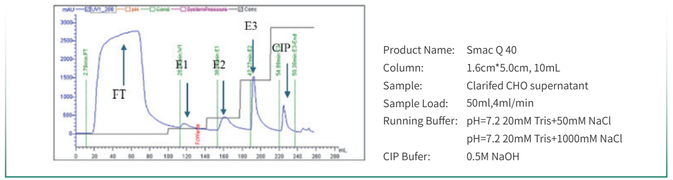


Figure 1. Strategy for RSV purification.



Product Name: Smac Q 40
Column: 1.6cm*5.0cm, 10mL
Sample: Clarifed CHO supernatant
Sample Load: 50ml,4ml/min
Running Buffer: pH=7.2 20mM Tris+50mM NaCl
pH=7.2 20mM Tris+1000mM NaCl
CIP Bufer: 0.5M NaOH

Figure 2.Q chromatographic curve.

Table 1. Specific Activity Assay Results.

Sample	ELISA(mg/ml)	A280 (mg/ml)	Relative Activity
Q-Loading	2.563	10.322	0.248
Q-Elution	0.984	0.663	1.484

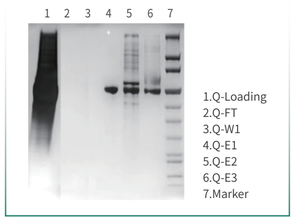


Figure 3.SDS-PAGE.

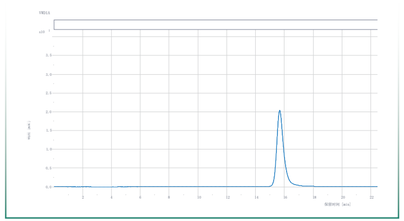
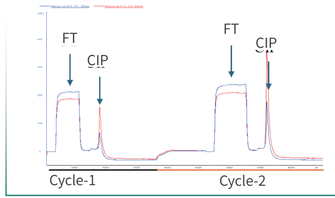


Figure 4.HPLC.

Chromatography effectively separates the target protein from impurities during the purification process. By optimizing elution conditions, high purity can be achieved, meeting registration and declaration requirements. The retained activity of the protein is confirmed through ELISA testing, yielding a final specific activity of 1.484. In the subsequent purification steps, CHT and HIC are employed to further reduce residual impurities, such as HCP and HCD, supported by excellent loading capacity and ultra-high resolution to ensure process robustness.

2) Whole-virus vaccines, a key component of traditional vaccines, play a significant role in the vaccine field due to their good safety profile. Whole-virus vaccines can be further classified into inactivated and live attenuated types. Molecular exclusion (Smartarose 4FF/6FF) and Multimode chromatography (Smart core 400/700)can be applied in the polishing of whole virus. At the same time, IEX chromatographic steps can enhance the overall process during development and manufacture phase. Smart-Lifesciences can provides complete solutions for whole virus vaccines and VLPs types.

Downstream Process of Rabies Vaccines



Product Name: Core 700
Column: 50cm*20cm, 392ml
Sample: Clarified Vero supernatant
Sample Load: 1CV & 2CV , 120cm/h
Running Buffer: pH=7.2 20mM PBS
CIP Bufer: 1M NaOH

Figure 5. Core700 Chromatographic Curve.

Table 2. Analysis Results.

	Volume (mL)	Antigen Activity (IU/ml)	Yield (%)	Content (ug/ml)	BSA (ug/ml)	HCP (ug/ml)	HCD (ug/ml)	Endotoxin (UE/ml)	Purity (%)
Sample	500	31.89	/	161	453	84.2	1-10	<2.5	95.1
FT	612	25.10	95.31	126	14	1.0	0.01-0.1	<0.25	99.3

After one-step Core 700 chromatography, the antigen yield exceeds 95%, and impurities in the bulk are effectively reduced, meeting the product's quality requirements. Additionally, compared to traditional Smartarose 4FF chromatography, this approach significantly increases sample loading volume and flow rate, thereby greatly enhancing purification efficiency.

Vaccines - Bacterial Antigens

Vaccines designed to target bacterial pathogens include polysaccharide vaccines and polysaccharide-conjugate vaccines, among which the purification of polysaccharides and tetanus toxin is a crucial component of bacterial vaccines. The following section presents the purification protocols for pneumococcal polysaccharide and tetanus toxin developed by Smart-Lifesciences.

1) Capsular polysaccharide is an important virulence factor of pneumococcal and serves as the main antigenic component of pneumococcal polysaccharide vaccine. The chemical structure and properties of capsular polysaccharides vary according to the serotypes of pneumonia, making purification methods a significant challenge. Type 5 capsular polysaccharide, a key component of pneumococcal carbohydrate vaccines, is especially difficult to purify due to high levels of impurities. By optimizing chromatographic conditions, Smart-Lifesciences has developed a suitable process route for the production of Type 5 polysaccharide, especially the performance of DEAE chromatographic resins in this project is very prominent.