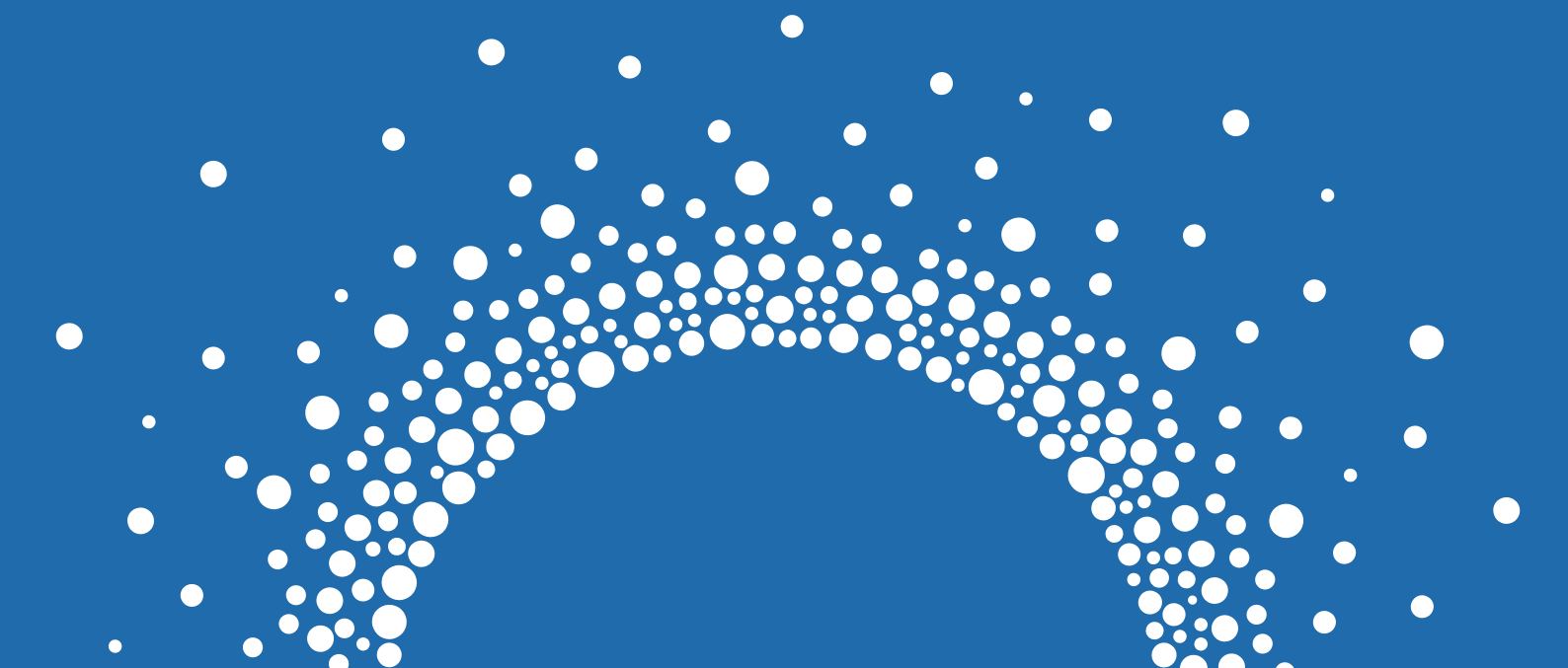
arProtein A Focurose HR

ANTIBODY PURIFICATION APPLICATION MANUAL

VDOBIOTECH

Healthy life starts here



COMPANY PROFILE

Located in the beautiful Suzhou Biomedical Industrial Park (BioBAY), which is highly consistent with BioBAY's positioning as an innovation base for bio-industry development, VDO has been developing rapidly over the years and has emerged as a leader in the biotechnology field with its diligent exploration in product development and rigorous and pragmatic attitude in management.

VDO was founded in 2014, and has been steadily exploring the deep value of the industry for many years, not only focusing on the scale production and application technology of microspheres, but also providing the overall solution of flow cytometry in clinical application and high quality in vitro diagnostic technology for life health industry, and successfully completed the transformation and industrialization of many cutting-edge innovative technologies.

With the mission of "Healthy Life Starts Here", VDO continues to promote technological innovation and lead the innovation and development of biomedical technology field. Looking ahead, VDO will continue to provide customers with high quality innovative products and high level solutions, and continue to contribute to the development and progress of biotechnology industry.

01 Corporate Culture

✓ Vision

To be a world-class biotech company

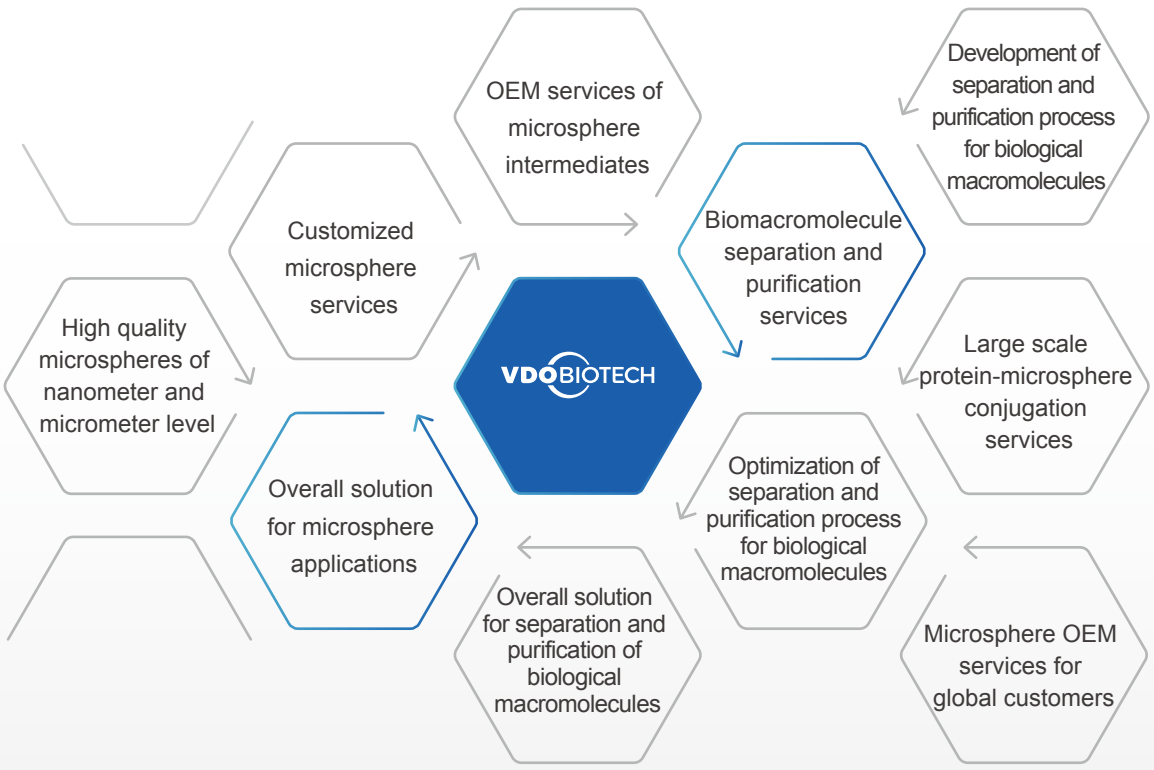
✓ Mission

Healthy life starts here

✓ Values

Presiseness Innovation Collaboration Openness

02 Service Items



RESIN INTRODUCTION

01 Resin Overview

Affinity resins were established and developed based on the principle of specific adsorption between biomolecules and other ligand molecules to achieve purification of target molecules by specific adsorption between the ligands on the medium and the target molecules. Protein A is a staphylococcus cell wall protein with a molecular weight of 42 KD, which contains five Fc segments that bind specifically to the Fc segment of antibody IgG molecules. It contains five structural domains that bind specifically to the Fc segment of the antibody IgG molecule.

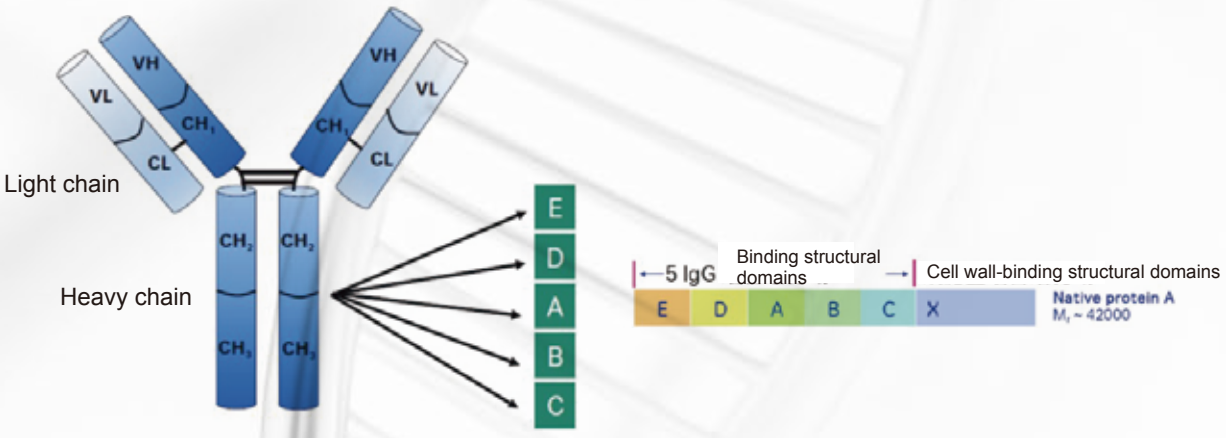
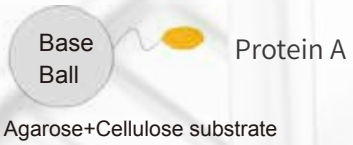


Fig. Binding domain of natural Protein A to Fc segment of IgG molecule

VDO has successfully developed arProtein A Focurose HR by linking its own modified Protein A ligand to cross-linked agarose. arProtein A Focurose HR is mainly used for the separation and purification of ascites, cell culture supernatants, serum-derived monoclonal antibodies, multiple antibodies and Fc-tagged proteins. The advantages of high loading capacity, stable alkaline resistance, excellent purification, good reproducibility, and direct substitution significantly reduce the customer's R&D and production costs.

02 Resin Structure



Agarose+Cellulose substrate

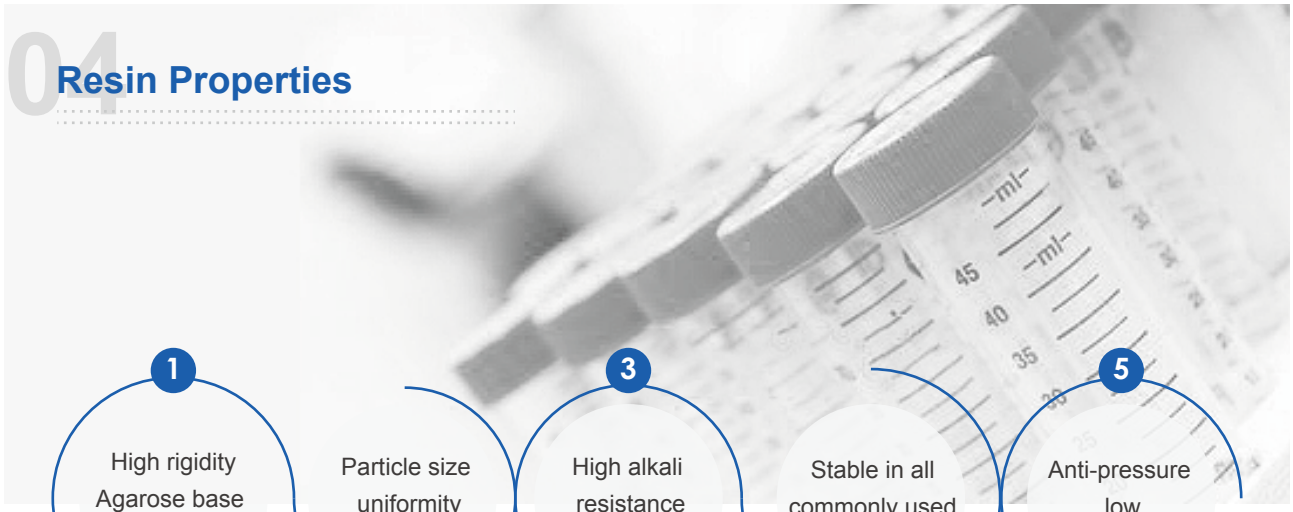
（Product graph）

03 Purification Process Of Monoclonal Antibody Captured By arProtein A Focurose HR Affinity Resin

Steps	Function	Column volume CV	Recommended solutions
Rinse1	Replacement of preservation fluid to prevent overpressure	2	20mM Tris-HCl, 150mM NaCl, pH7.4
Pre-disinfection	Ensure the chromatographic column is clean before use	3	0.1M-0.5M NaOH
Balance	Equilibrium chromatography column, ready for sample loading	3	20mM Tris-HCl, 150mM NaCl, pH7.4
Sampling	Capture of target proteins	Calculation based on packing capacity	Cell culture clarified fermentation broth
Rinse1	Unbound proteins in the top wash system	3	20mM Tris-HCl, 150mM NaCl, pH7.4
Rinse2	Removal of non-specific binding impurities	3	20mM Tris-HCl, 1M NaCl, pH7.4
Rinse3	Displacement buffer system for subsequent elution	3	50mm NaAc-HAc, pH5.5
Elution	Dissociation of target proteins	3	50mm NaAc-HAc, pH3.6
Regeneration	Cleaning of over-bonded impurities in the packing	3	1M HAc
Rinse2	Displacement buffer system for subsequent disinfection	2	20mM Tris-HCl, 150mM NaCl, pH7.4
Post-sterilization	Ensure the chromatographic column is clean after use	3	0.1M-0.5M NaOH
Rinse3	Displacement buffer system for subsequent preservation	2	20mM Tris-HCl, 150mM NaCl, pH7.4
Storage	Save filler for next use	3	20% Ethanol

Note: If you need to repeat the sample, go directly from the "post-sterilization" step to the "balance" step.

04 Resin Properties



1. High rigidity Agarose base holder
2. Particle size uniformity ~75μm
3. High alkali resistance 0.5M NaOH
4. Stable in all commonly used buffers
5. Anti-pressure low

05 Technical Parameter

Resin	Highly rigid agarose
Average particle size D50	~75μm
Dynamic binding capacity (DBC)	~60mg(human IgG) /mL； Test parameters: 5min retention time, column height 10cm
pH stability	3-12(long term) 2-14(short term)
Operating Temperature	4°C-40°C
Recommended retention time	4-6min, specific retention time can be adjusted according to project requirements
Maximum flow rate	500cm/h
Resin tolerance pressure	≤0.5MPa
Storage fluid	20% ethanol or 2% benzyl alcohol
Storage conditions	2°C-8°C

Note: (1) The binding load of the resin will vary depending on the source and isotype of the sample.
(2) Resins that are immersed in the eluent for a long time will lead to hydrolysis of the ligand and eventually affect the resin loading.

06 Performance Characteristics

High DBC loading

The average dynamic binding load ＞ 60 mg/mL, and the recommended retention time was 5 min.

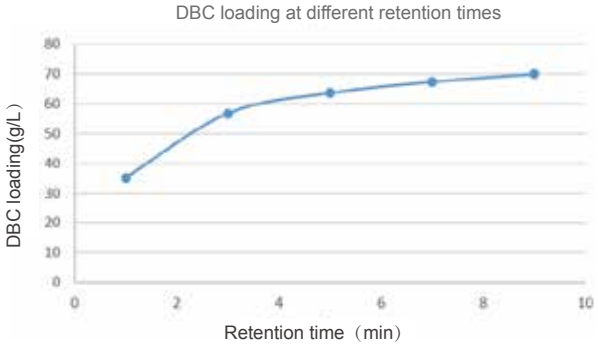


Fig. 2 Dynamic loading with different retention times

Anti-low pressure

At the same column height and linear flow rate, the backpressure of our R&D type columns is comparable to that of production grade columns.

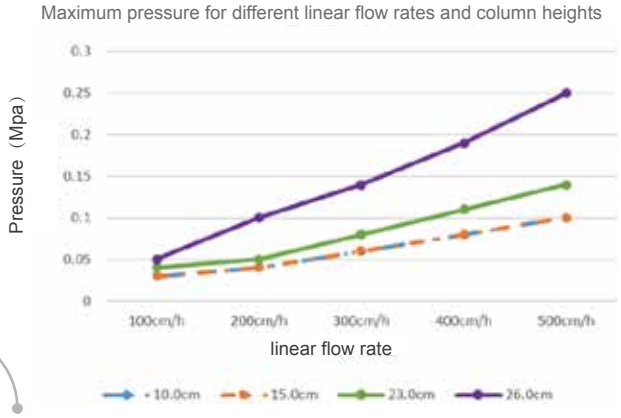


Fig. 1 arProtein A Focurose HR pressure flow rate test

The test was conducted using a 1.6 cm diameter column, and the pressure was around 0.25 MPa at a column height of 26 cm and 500 cm/h, which is far below the maximum pressure resistance of 0.5 MPa for this resin.

Strong alkali resistance (static)

0.1M NaOH immersion for 1440h, DBC load was not affected; 0.5M NaOH, 1M NaOH immersion for 168h, DBC load decreased <20%.

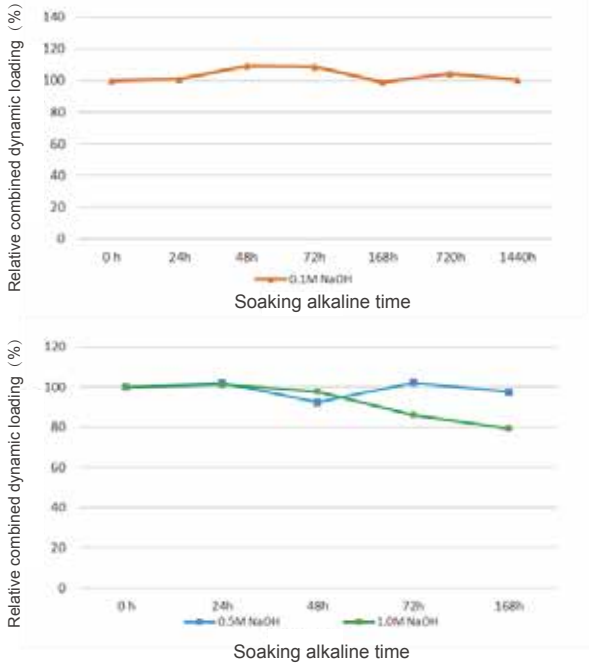


Fig. 3 arProtein A Focurose HR static alkaline resistance test

Strong alkali resistance (dynamic)

0.1M NaOH 200 cycles DBC remained essentially unchanged; 0.5M NaOH 100 cycles DBC was maintained at 80% of the initial value.

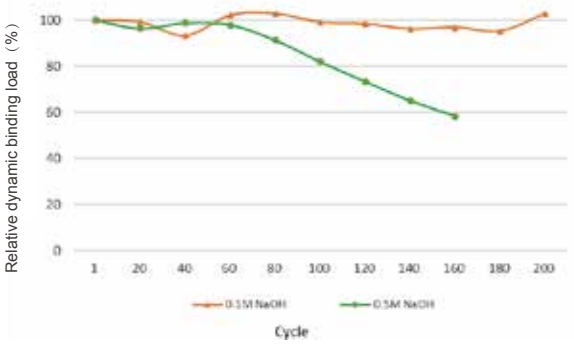


Fig. 4 arProtein A Focurose HR dynamic alkaline tolerance test

Normal treatment with 0.1M NaOH for 15-30min;
When thorough disinfection is required, 0.5M NaOH treatment can also be used for 15-30min.

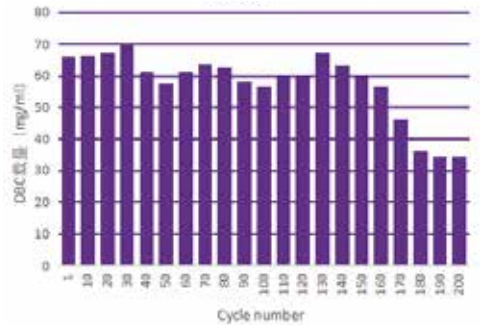
07 Life Verification

01

High DBC loading

0.1M NaOH was used before and after the single experiment to disinfect for 15 min (equivalent to after 400 alkaline washes); The DBC loading was kept at about 60 mg/mL, and the percentage of decrease within 160 times within 15%.

DBC loading

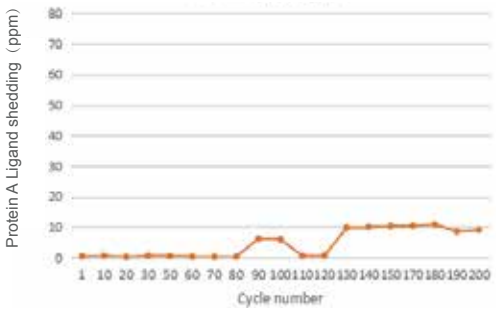


02

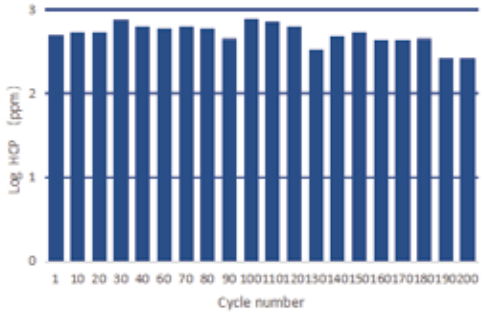
Low ligand shedding

The residue of Protein A ligand was less than 10 ppm in 200 times of monoclonal antibody lifetime validation, and there was no significant loss of ligand within 80 times of use.

Protein A Ligand shedding



CHO HCP remove



Low host cell protein (HCP) residues

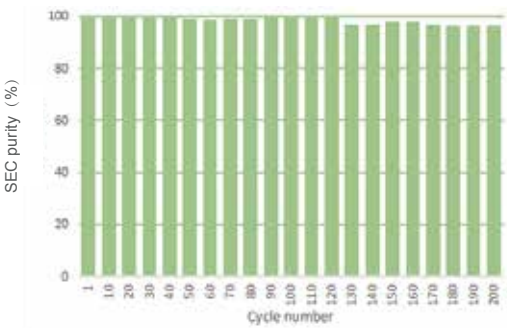
The HCP residues were all within 800 ppm in 200 times of single antibody life validation, which was consistent with imported resins.

03

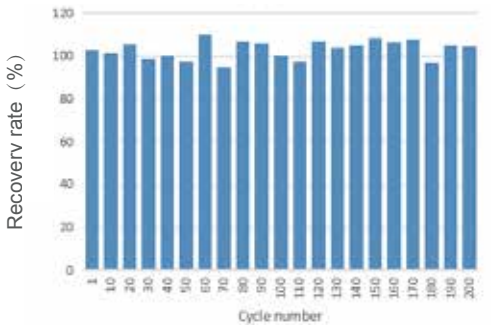
High purity

The purity of SEC was maintained above 95% in 200 lifetime validations of the monoclonal antibodies, indicating that the filler is highly stable in terms of purity enhancement.

SEC purity



Antibody recovery rate



High recovery rate

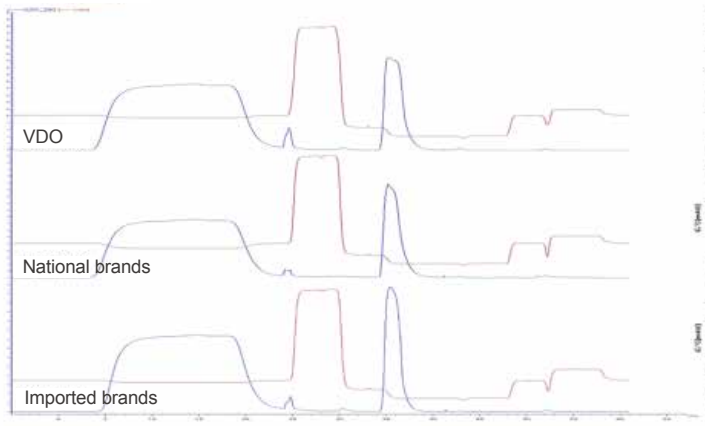
The recovery rate was greater than 94%. The recovery rate did not decrease significantly over the lifetime of the 200 monoclonal antibodies, and was essentially within 10%.

08 Application Case

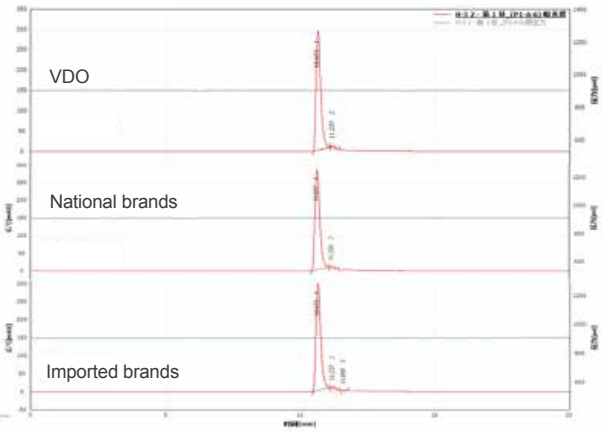
Case 1: comparison of arProtein A Focurose HR with other brands of packed purified CHO cell fermentation monoclonal antibody

The VDO arProtein A Focurose HR filler was compared with imported and national brands of purified monoclonal antibodies, and the results showed that the antibody recovery was 94.84% better than that of imported and national brands. The purity was not significantly different and was around 98.4%.

a.Chromatograms

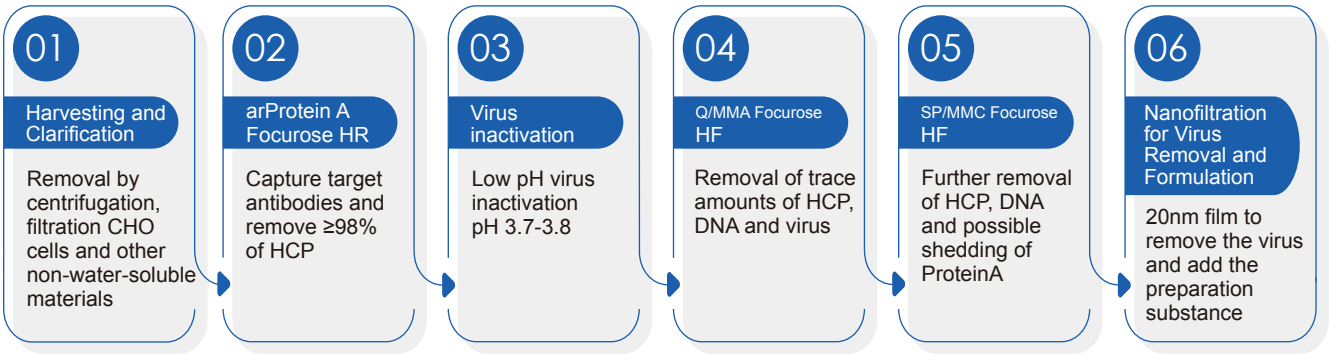


b. HPLC-SEC



Brands	Antibody recovery rate	Purity
VDO	**94.84%**	98.43%
National brands	91.79%	98.49%
Imported brands	90.78%	98.48%

ANTIBODY PURIFICATION PROCESS



ORDERING INFORMATION

01 Affinity Resins

Product name	Spec	Product number
arProtein A Focurose HR	25mL	HQ320827025M
	100mL	HQ320827100M
	500mL	HQ320827500M
	1L	HQ320827001L
	5L	HQ320827005L
	20L	HQ320827020L

02 Ion Exchange Resins

Product name	Spec	Product number
Q Focurose HF	25mL	HL280306025M
	100mL	HL280306100M
	500mL	HL280306500M
	1L	HL280306001L
	5L	HL280306005L
	20L	HL280306020L

Product name	Spec	Product number
SP Focurose HF	25mL	HL280301025M
	100mL	HL280301100M
	500mL	HL280301500M
	1L	HL280301001L
	5L	HL280301005L
	20L	HL280301020L

03 Hydrophobic Interaction Resins

Product name	Spec	Product number
Butyl Focurose 4FF	25mL	HS030306025M
	100mL	HS030306100M
	500mL	HS030306500M
	1L	HS030306001L
	5L	HS030306005L
	20L	HS030306020L
Phenyl Focurose HP	25mL	HS060202025M
	100mL	HS060202100M
	500mL	HS060202500M
	1L	HS060202001L
	5L	HS060202005L
	20L	HS060202020L
Phenyl Focurose FF(LS)	25mL	HS060301025M
	100mL	HS060301100M
	500mL	HS060301500M
	1L	HS060301001L
	5L	HS060301005L
	20L	HS060301020L

Product name	Spec	Product number
Butyl Focurose HP	25mL	HS060206025M
	100mL	HS060206100M
	500mL	HS060206500M
	1L	HS060206001L
	5L	HS060206005L
	20L	HS060206020L
Phenyl Focurose FF(HS)	25mL	HS060302025M
	100mL	HS060302100M
	500mL	HS060302500M
	1L	HS060302001L
	5L	HS060302005L
	20L	HS060302020L

04 Multimodal Resins

Product name	Spec	Product number
MMA Focurose HF	25mL	HF190309025M
	100mL	HF190309100M
	500mL	HF190309500M
	1L	HF190309001L
	5L	HF190309005L
	20L	HF190309020L
MMC Focurose HF	25mL	HF190305025M
	100mL	HF190305100M
	500mL	HF190305500M
	1L	HF190305001L
	5L	HF190305005L
	20L	HF190305020L

Product name	Spec	Product number
MMA Focurose HR	25mL	HF190809025M
	100mL	HF190809100M
	500mL	HF190809500M
	1L	HF190809001L
	5L	HF190809005L
	20L	HF190809020L

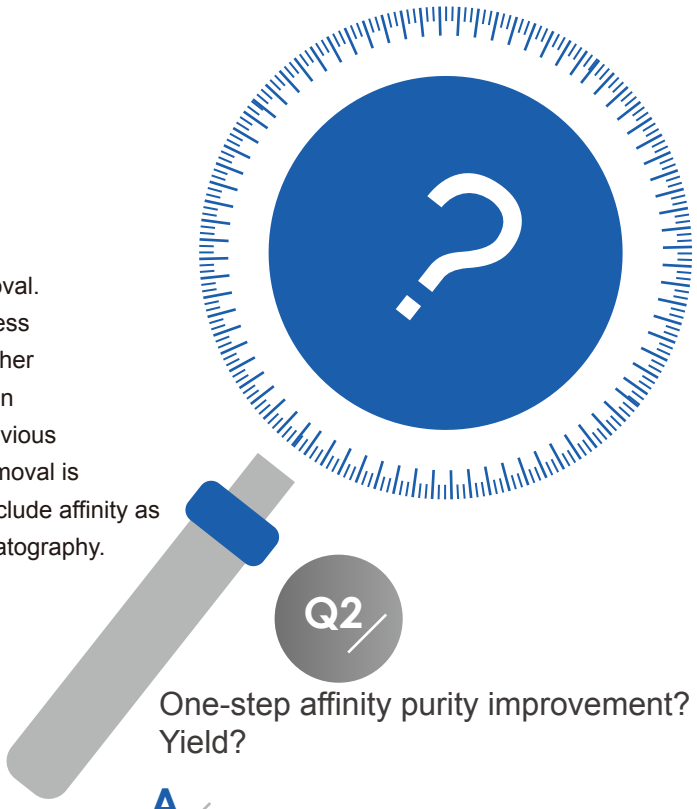
RELEVANT Q&A

Q1

One-step purified virus clearance?

A

For affinity resins, there are no fixed value for virus removal. However it generally depends on the sample itself, process conditions and incubation conditions, and in general, higher non-specific adsorption of proteins or higher glycosylation will reduce virus removal. Based on experience from previous monoclonal antibody projects, the Log value for virus removal is between 1-3. For virus removal validation, it is safe to include affinity as a step in the final calculation for virus removal by chromatography.



Q2

One-step affinity purity improvement? Yield?

A

In general, the purity improvement will reach more than 95% and the yield will be more than 90%. It depends on the project and the specific situation.

Q3

Recommended column efficiency range? Column efficiency measurement method?

A

For 0.66cm-1.1cm diameter columns with a column height of 10cm or more, the column efficiency should meet 1000N/m or more, and the symmetry should be between 0.8-1.6.

For 1.6cm-5.0cm diameter columns with a column height of 10cm or more, the column efficiency should meet 2000N/m or more, and the symmetry should be between 0.8-1.6.

For larger column, the symmetry of column efficiency can be specified according to customer requirements, generally speaking the larger the column the higher the column efficiency will be.

Generally speaking, the larger the column, the higher the column efficiency. The production scale generally has a column efficiency above 4000N/m and a symmetry of 0.8-1.6.

There are two ways to determine the column efficiency, water and 1% acetone for UV determination, or 0.4M NaCl and 0.8M NaCl for conductivity determination. The measurement method is 100cm/h linear flow rate with 2% column volume injection.

Q4

What are the reasons for the forward tilt and tailing when measuring column efficiency? Does it have any effect on purification?

A

When measuring the column effect, the forward tilt is generally due to the packing pressure is too tight, or the packing solid-liquid ratio is too high.
When measuring the column effect occurs trailing is generally due to the packing pressure is too loose, or packing solid-liquid ratio is too low caused by.
For the impact of purification: the peak shape of the column effect directly reflects the peak shape of the later purification, either forward or tailing will lead to inaccurate collection of target product peaks. Purification chromatography column for the symmetry of the provisions, generally within the prescribed range to ensure the rigor of the experiment.

Q5

Recommended concentration of the sample?

A

The loading concentration is generally recommended to be 0.8-5 g/L. Too low or too high a concentration will cause unsatisfactory loading and purification results;
According to the DBC results, the maximum binding load of the filler is between 60-70 g/L. In order to ensure the yield and purification effect, the recommended loading volume is between 20-50 g/L.

Q6

Recommended buffer system

A

The equilibrium solution is usually a neutral buffer, commonly used are phosphate system and Tris-HCl system.
The eluent is generally recommended to be acidic buffer, commonly used are acetic acid-sodium acetate system, citric acid-sodium citrate system, glycine-hydrochloric acid system, etc. PH3.5-3.8 is used more often.

Q7

Filler disinfection?
Conditions of alkali?

A

Normal treatment with 0.1M NaOH for 15-30min.
When thorough disinfection is required, 0.5M NaOH treatment can also be used for 15-30min.

Healthy Life Starts Here

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