

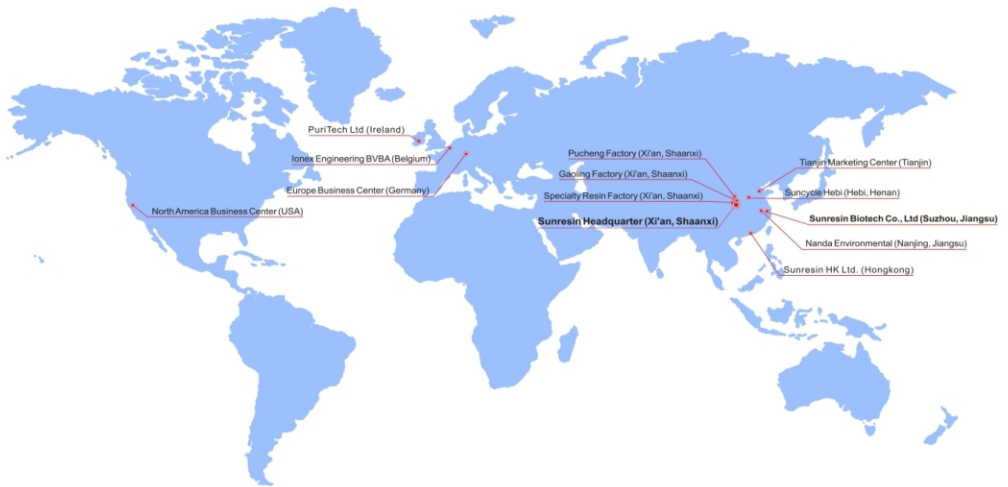
Seplife Chromatography Resins

[2022Version]



SEPLIFE

- Selection Guide of Chromatography Resins
- Gel Filtration Chromatography Resins (GF)
- Ion Exchange Chromatography Resins (IEX)
- Hydrophobic Interaction Chromatography Resins (HIC)
- Affinity Chromatography Resins(AC)
- Multimodal Chromatography Resins (MM)
- HM Chromatography Resins
- Microcarriers for Cell Culture



Sunresin New Materials Co. Ltd.

Sunresin is an innovation oriented high-tech enterprise, specializing in supplying ion exchange resins, adsorption and chromatography resins, equipment solutions and relevant technical services. With 20 years of manufacturing experience, it is the only listed company in the IER industry (Shenzhen Stock code 300487) in China. Sunresin has 8 subsidiaries, 3 overseas branch offices, as well as 5 state-of-the-art manufacturing facilities around the world. Sunresin produces over a hundred resin products covering a wide range of applications for separation and purification. Sunresin's products portfolio includes about 30 product categories and more than 100 different resin references which are commercialized in over 100 countries and broadly used in industries such as Water and wastewater treatment, Food Processing, Biotech, Pharmaceuticals, Plant Extraction, Membrane Caustic Soda, Hydrometallurgy, as well as Municipal Water Treatment among others.

Sunresin focuses on innovation, quality and services. Sunresin holds more than 30 patents, in China and internationally, and has accomplished more than ten national projects in the area of resin development. Sunresin is certified under ISO 9001 for Quality Control System and ISO14001 for Environment Control System. It has also been awarded with Certificates from WQA Golden Seal, Kosher, CE, Halal, etc. Under worldwide recognized QC systems, Sunresin provides excellent and high quality products to the market. All of the employed manufacturing processes are strictly controlled by the environmental regulations.

Based on the technical competence, the rich experience and under strict international standards, Sunresin supplies high quality products at a fast delivery time, cost-effective equipment, professional design and solution, as well as proactive customer services to our customers.



Administration & R&D



Manufacturing



Suzhou Sunresin Biotech Co., Ltd

Suzhou Sunresin Biotech Co., Ltd, a wholly-owned subsidiary of Sunresin New Materials Co., Ltd (Stock Code: SZ300487), is a leading high-tech company specializing in the R&D and production of chromatography resins for the downstream separation and purification process of biomedicines, cell culture microcarriers, solid-phase synthesis carriers (polypeptides and nucleic acid) as well as immobilized enzyme carriers. The company is also a solution provider for process development of the chromatography systems for customers.

Located in the Suzhou Industrial Park (SIP), Sunresin Biotech leverages its parent company's technology platform in developing agarose, dextran and polymer-matrix resins and rich industrial purification experiences to provide purification products and materials to the pharmaceutical and biotech industries. It is committed to becoming China's leading chromatography resins supplier and pushing the industry to a new height around the world.

• Standardized Products

Sunresin has a complete production line for agarose-matrix, dextran-matrix, and polymer-matrix chromatography resins, with an annual production capacity of 50,000L in total. The product quality is stable and has reached the international leading level.

• Core competitiveness

The key technicians of Sunresin have nearly 20 years of experience in the development, production and application of agarose matrix, dextran matrix and polymer matrix chromatography resins. With rich product categories and reliable performance, we can also provide customized services for specific products and requirements of customers on the basis of standardized products, and develop tailored products not only to meet the customers' needs but also help them enhance their competitiveness in the market through optimized process.

• Strict Product Quality Control

Sunresin has invested significantly in world-leading top testing instruments and has established its own quality management system and norms in strict accordance with GHS, ISO9001 and the guidelines of pharmaceutical production related management norms. From raw materials to production process control and finished product testing, all methods and processes are performed in strict accordance with the documents to ensure stable product quality and provide guarantee for the safety and stability of the products.

• Solution Provider in Process Development

Sunresin can design and develop a product purification process that meets future production and linear scale-up requirements by combining cell culture methods and separation and purification technology with the performance of our chromatography resins. We can also work with customers to optimize their original production process to improve stability, increase yield and reduce cost.

• Stable Automated Production Control

Sunresin has developed its own world-leading automated console system to monitor the production operation in real time and avoid the influence of human impacts on product quality to the maximum extent, leading to a guaranteed reproducibility and stability of the products.



Acronyms and Abbreviations:

Seplife : Trademark of Sunresin

6FF: Fast Flow, 6% high flow rate agarose matrix, particle size range 45-165 μ m, average particle size 90 μ m

4FF: Fast Flow, 4% high flow rate agarose matrix, particle size range 45-165 μ m, average particle size 90 μ m

BB: Big Beads, 6% large particle high flow rate agarose matrix, particle size range 100-300 μ m, average particle size 200 μ m

HP: High Performance , 6% high resolution agarose matrix, particle size range 25-44 μ m, average particle size 34 μ m

XL: 6% High flow rate, high capacity agarose matrix, particle size range 45-165 μ m, average particle size 90 μ m

Large Scale: High rigidity agarose matrix, particle size range 45-165 μ m, average particle size 90 μ m

Large Scale HP: High rigidity and high resolution agarose matrix, particle size range 25-44 μ m, average particle size 34 μ m

LS: Low Sub

HS: High Sub

SF: Superfine particle size, dextran matrix, dry powder particle size 20-50 μ m

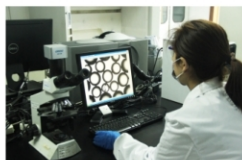
F: Fine particle size, dextran matrix, dry powder particle size 20-80 μ m

M: Medium particle size, dextran matrix, dry powder particle size 50-150 μ m

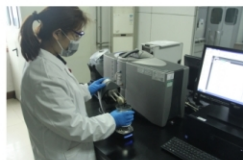
C: Coarse particle size, dextran matrix, dry powder particle size 100-300 μ m

cm/h: Linear flow rate (cm/h) = flow rate (ml/min) x 60 / (π x column radius (cm) ²)

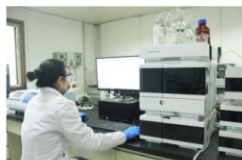
Quality Control and Performance Evaluation at Sunresin



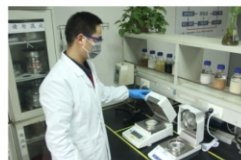
Electron microscope - morphology analysis



Malvern laser analyzer - particle size analysis



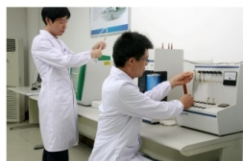
Liquid chromatography- reagent residue analysis



Water content measurement



ICP - Heavy metal content test



Pore volume, pore size measurement



Automatic acid-base titrator



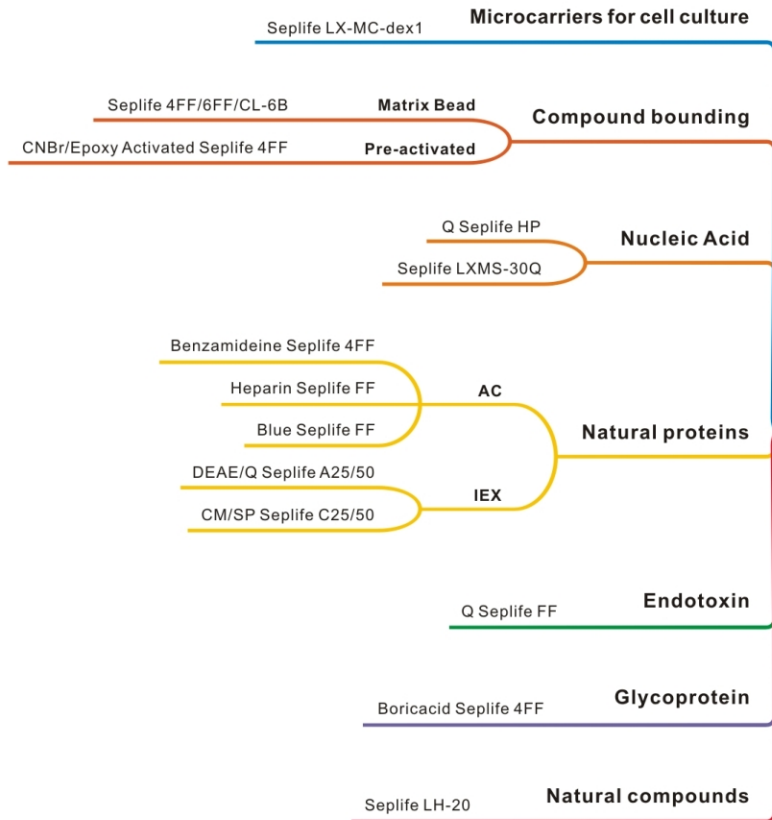
Fourier-transform infrared spectroscopy- structural analysis

Table of Contents



1. Selection Guide of Seplife Chromatography Resins	01 / 04
2. Gel Filtration Chromatography Resins (GF)	05 / 10
2.1 Agarose GF Resins	06
2.2 Dextran GF Resins	08
2.3 Allyl Dextran GF Resins	10
3. Ion Exchange Chromatography Resins (IEX)	11 / 16
3.1 High Flow Rate Agarose IEX resins	11
3.2 High Capacity Agarose IEX resins	13
3.3 Large Scale Agarose IEX resins	13
3.4 Dextran IEX resins	15
4. Hydrophobic Interaction Chromatography Resins (HIC)	17 / 19
5. Affinity Chromatography Resins	20 / 26
5.1 Metal Chelate Affinity Resins	20
5.2 Affinity Resins for Purification of GST- Tagged Proteins	22
5.3 Affinity Resins for Antibody Purification	22
5.4 Affinity Resins for Plasmid DNA Purification	23
5.5 Affinity Resins for Serine Protease Purification	23
5.6 Heparin Affinity Resins	24
5.7 Boric Acid Affinity Resins	25
5.8 Blue Dye Affinity Resins	25
5.9 Red Dye Affinity Resins	26
5.10 Pre-activated Chromatography Resins	26
6. Multimodal Chromatography Resins (MM)	27 / 29
6.1 Seplife Suncore 700 Multimodal Resins	27
6.2 MA/MMC Seplife Multimodal Resins	28
6.3 Seplife LH-20 Multimodal Resins	29
7. HM Chromatography Resins	30 / 32
8. Microcarriers for Cell Culture	33
9. Annexes	34 / 38

Selection Guide of Seplife Chromatography Resins



Selection Guide for Seplife

Chromatography Resins

Tagged recombinant proteins

- HIS Tag** Ni Seplife FF/NTA/TED
- GST Tag** Glutathione Seplife 4FF
- Fc Fusion** rProtein A Seplife Suno

Viral biological products

- MM** Seplife Suncore 700
- GF** Seplife 4FF/6FF
- IEX** Q/SP Large Scale
- HIC** Butyl Seplife 4FF
- AC** Ab+CNBr Seplife 4FF

Desalting and buffer exchange

Seplife G-25M

Antibodies

- AC** rProtein A Seplife Suni/Suno
- GF** Seplife S-100/S-200
- IEX** Q/SP Large Scale
- HIC** Phenyl Seplife FF
- MM** MA/MMC Large Scale

Inclusion body proteins

- GF** Seplife S-100/S-200
- IEX** Q/SP Seplife FF
- HIC** Phenyl/Butyl Seplife FF
- AC** Ni Seplife FF NTA

Sample after salting out with Ammonium sulfate

- Phenyl/Butyl Seplife FF
- DEAE/CM/Q/SP Seplife XL

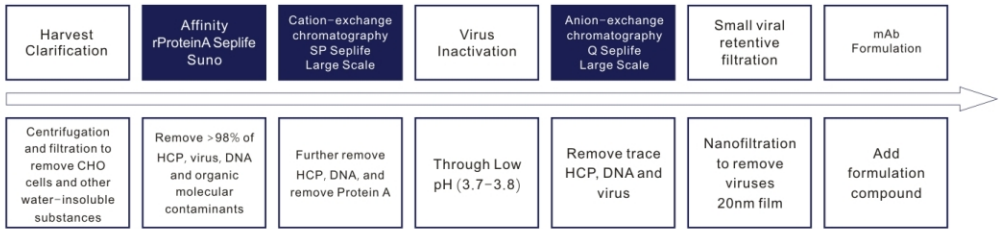
mRNA

- Q Large Scale HP
- Large Scale Oilgo dT

Plasmid

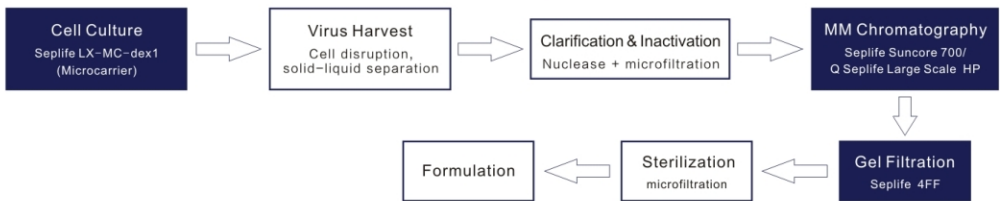
- Seplife 6FF
- Seplife Plasmid XL
- Seplife LXMS-30Q
- Pheny Seplife FF
- Seplife Suncore 700

Purification Process for Therapeutic Monoclonal Antibodies

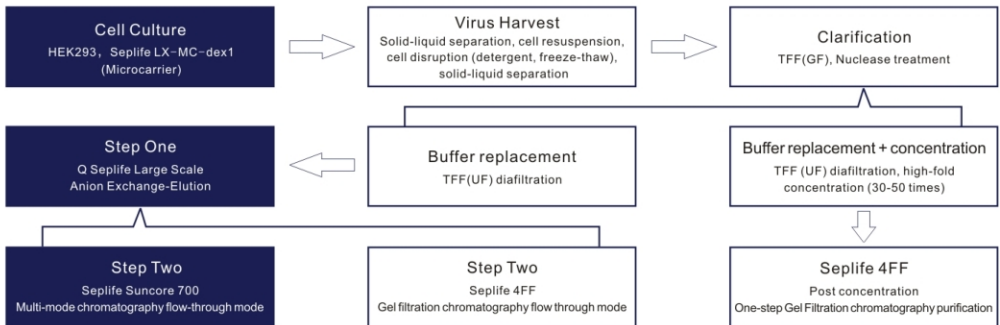


Purification Process for Inactivated Vaccines

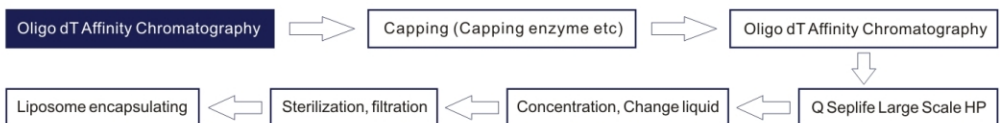
Downstream purification process of COVID-19 Inactivated Vaccines



Purification Process for Adenovirus Vector Vaccines

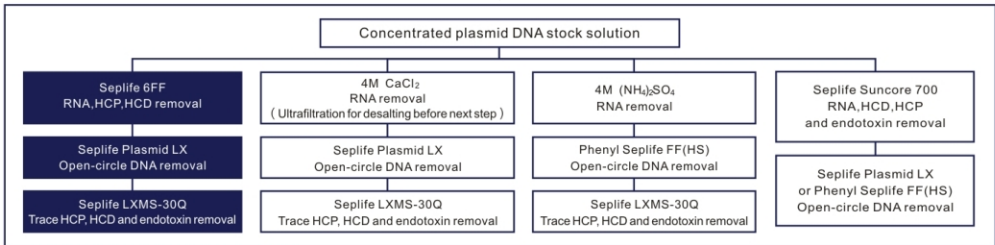
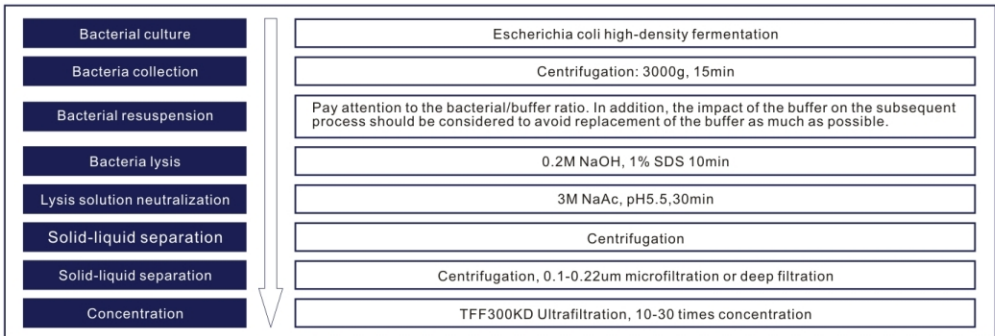


Purification Process for mRNA Vaccines

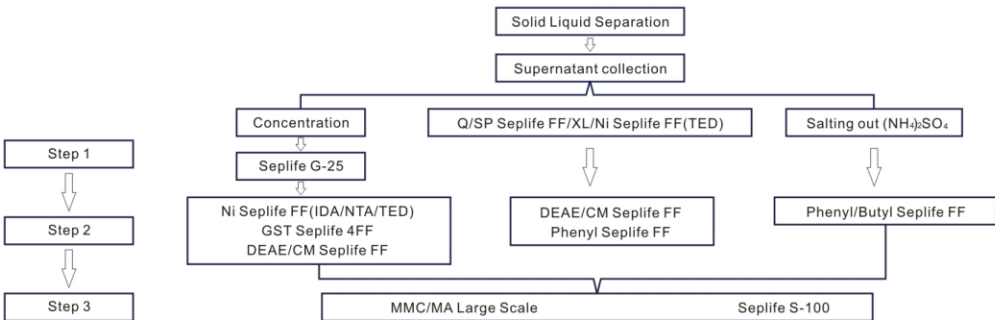


Note: Every time when proceeding to the next operation process after one operation, the applicability of the buffer and the mRNA concentration must be considered, to determine whether it is necessary to adjust the mRNA concentration and the buffer system by means of ultrafiltration concentration and replacement of the buffer.

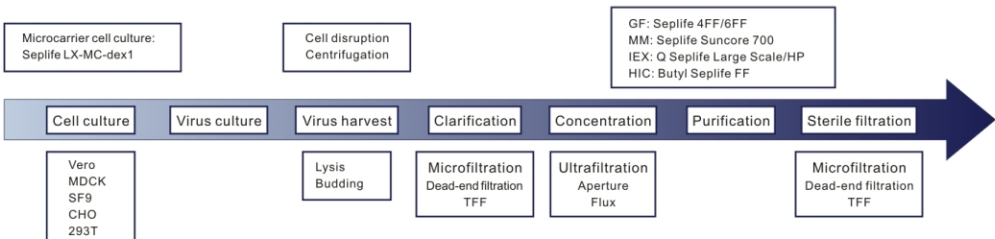
Large-scale preparation of plasmid DNA



Purification process for recombinant proteins



Preparation process of virus and virus vector





SEPLIFE

Gel Filtration Chromatography Resins (GF)

Gel Filtration chromatography is a chromatographic method to achieve separation based on the difference in the size of biomolecules. It is also called size exclusion chromatography.

The gel filtration resins are a kind of three-dimensional mesh structure spherical media with different pore size distribution. When the biomolecules of different morphological sizes flow through the gel filtration resins, the different pore size and distribution of the resins make the biomolecules of different sizes have different retention times, thus realize the separation of biomolecules of different sizes.

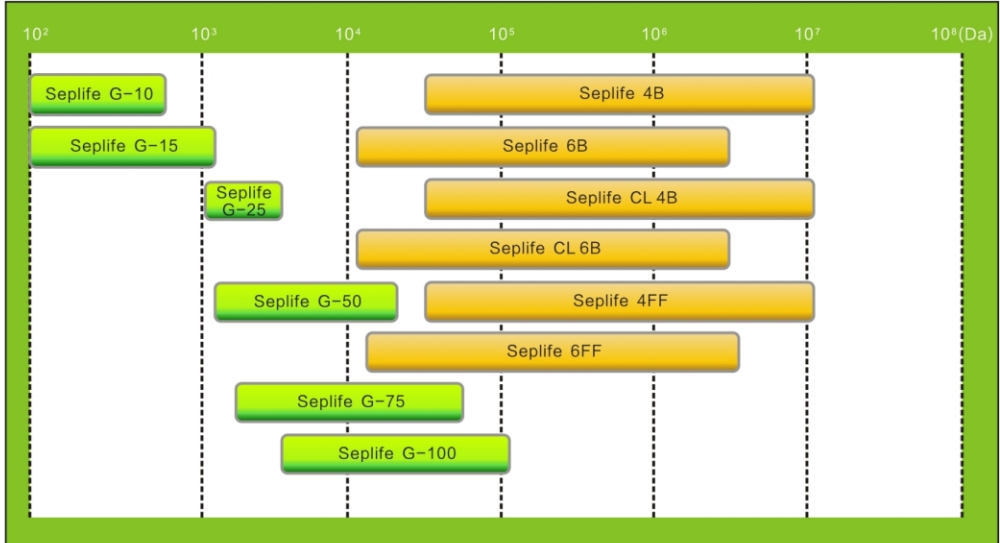
• Characteristics of Gel Filtration

1. Separation principle understandable and simple to operate.
2. Inert media with very low non-specific adsorption.
3. Mild separation conditions, high recovery of target protein.
4. The presence of ions, small molecule impurities, decontaminants, etc. in the mobile phase do not affect the separation.
5. Wide pH range

• Main applications of Gel Filtration

1. Desalting and buffer exchange. The sample volume can reach 25-30% of the column bed volume
2. Separation of proteins of different molecular sizes, removal of virus particles and aggregates. The sample volume is generally 1-5% of the column volume, and the height of the column bed is required to be ≥ 60 cm.

• Molecular size range of Seplife Gel Filtration Resins' separation



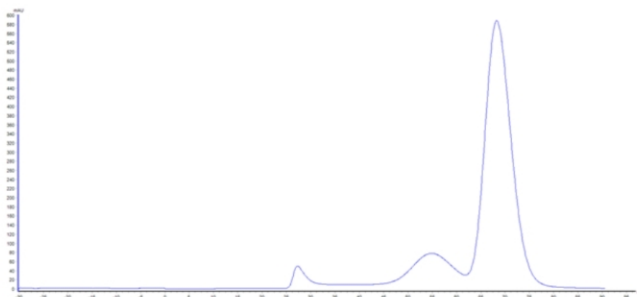


Agarose Gel Filtration Resins

Because of the different agarose content in the synthesis process of agarose gel filtration resins, micellar pores with different size distributions are produced, resulting in different molecular size separation. Currently, two widely used agarose gel filtration resins Sunresin produces are Seplife 4B and Seplife 6B with 4% and 6% agarose. The internal molecular structure of Seplife 4B and Seplife 6B gel filtration resins is fixed by hydrogen bonding and the matrix is soft and has limited pressure resistance. In order to meet the needs of industrial-scale gel filtration separation and purification, to improve the structural rigidity of the resins and to increase the processing flow rate and pressure resistance, the Seplife 4B and Seplife 6B resins have been cross-linked once or repeatedly to produce the cross-linked agarose series Seplife CL-4B and Seplife CL-6B as well as the fast flow rate agarose Seplife 4FF and Seplife 6FF resins with better pressure resistance.

• Technical parameters of Agarose GF resins

Product	Exclusion Range (globular proteins), Da	Particle size (μm)	Max. Flow rate (cm/h)	Max. Pressure (MPa)	pH Stability	Chemical Resistance
Seplife 4B	60000-20×10 ⁶	45-165 μm	120	0.1	4-9 (Operational) 3-11 (CIF)	Resistant to 8mol/L urea, 6mol/L guanidine hydrochloride
Seplife 6B	10000-4×10 ⁶		180	0.1		
Seplife CL 4B	60000-20×10 ⁶		180	0.12	3-13 (Operational) 2-14 (CIF)	Resistant to 8mol/L urea, 6mol/L guanidine hydrochloride; organic solvents such as ethanol, DMF, THF, DMSO, CH ₃ Cl, acetone, dimethylformamide, methylene chloride, pyridine, acetonitrile
Seplife CL 6B	10000-4×10 ⁶		240	0.12		
Seplife 4FF	60000-20×10 ⁶		420	0.3	2-12 (Operational) 2-14 (CIF)	Stable at 40°C in the following solutions: 2mol/L NaOH; 70% EtOH; 30% isopropanol; 30% acetonitrile; 1% SDS; 6mol/L guanidine hydrochloride; 8mol/L urea
Seplife 6FF	10000-4×10 ⁶		750	0.3		



Column: XK16/70

Chromatography resin: Seplife 6FF

Sample: 1. Blue glucose (Mr 2, 000,000)

2. BSA (Mr 67,000)

3. cytidine (Mr 243)

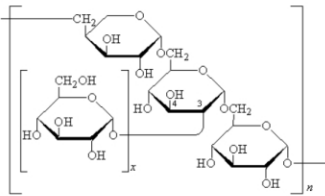
- Ordering information for agarose gel filtration resins

Product	Ref. No.	Pack Size
Seplife 4B	A1001102	25ml
	A1001103	100ml
	A1001104	500ml
	A1001105	1L
	A1001106	5L
	A1001107	10L
Seplife 6B	A1001202	25ml
	A1001203	100ml
	A1001204	500ml
	A1001205	1L
	A1001206	5L
	A1001207	10L
Seplife CL4B	A1002102	25ml
	A1002103	100ml
	A1002104	500ml
	A1002105	1L
	A1002106	5L
	A1002107	10L

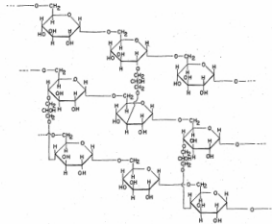
Product	Ref. No.	Pack Size
Seplife CL6B	A1002202	25ml
	A1002203	100ml
	A1002204	500ml
	A1002205	1L
	A1002206	5L
	A1002207	10L
Seplife 4FF	A1003102	25ml
	A1003103	100ml
	A1003104	500ml
	A1003105	1L
	A1003106	5L
	A1003107	10L
Seplife 6FF	A1003202	25ml
	A1003203	100ml
	A1003204	500ml
	A1003205	1L
	A1003206	5L
	A1003207	10L

Dextran Gel Filtration Resins

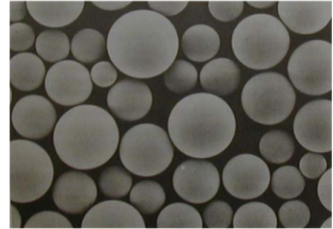
Sunresin's Seplife G dextran gel filtration resins are hydrophilic inert media with a certain pore size distribution, synthesized from specially selected dextran and cross-linked by cross-linking agents. The degree of cross-linking during the synthesis of the resins is controlled to produce different swelling properties and pore size distributions, resulting in differences in the molecular size range of the separation. Seplife G dextran Gel Filtration resins are used in a wide range of industrial applications such as buffer exchange, desalting, removal of small molecules and separation of substances of different molecular sizes.



Dextran Structure



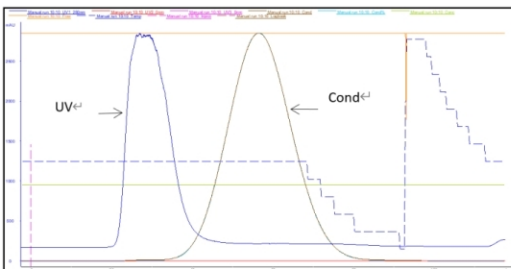
Dextran GF Structure



Dextran GF beads under microscope (270:1)

• Technical Parameters of Seplife Dextran Gel Filtration Resins

Products	Particle Size (dry μm)	Swelling Property (ml/g)	Exclusion range (globular proteins Da)	Max. Flow rate (cm/h)	pH stability	Max. pressure (MPa)
Seplife G25 C	100-300	4-6	100-5000	480	3-10 (Operational) 2-13 (CIF)	The flow rate is proportional to the pressure, according to Darcy's Law
Seplife G25 M	50-150					
Seplife G25 F	20-80					
Seplife G25 SF	20-50					
Seplife G50 C	100-300	9-11	$1500-3 \times 10^4$	180		
Seplife G50 M	50-150					
Seplife G50 F	20-80			60		
Seplife G50 SF	10-40	12-15	$3000-8 \times 10^4$	90		
Seplife G75 M	40-120			50		
Seplife G75 SF	10-40	15-20	$3000-7 \times 10^4$	90		
Seplife G100 M	40-120					
Seplife G100 SF	10-40		$4000-15 \times 10^4$	30		



Chromatography column: XK26/20;

Resin: Seplife G25 M;

Sample volume: 50% of column volume;

Flow rate: 120 cm/h

- Ordering information for Seplife dextran gel filtration resins

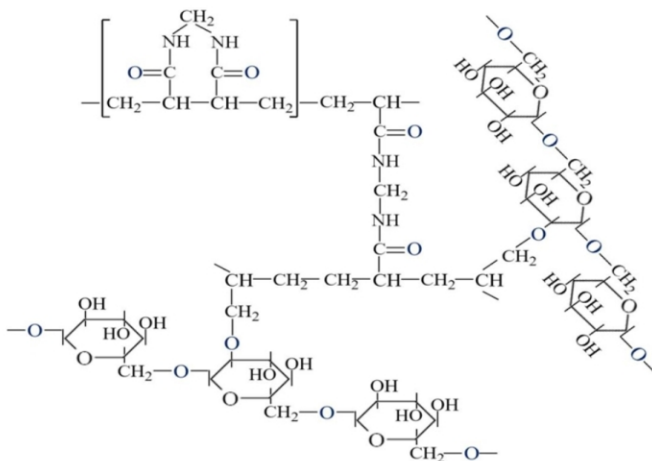
Product	Ref. No.	Pack Size
Seplife G25 C	D1007210C	25g
	D1007211C	100g
	D1007212C	500g
	D1007213C	1kg
	D1007214C	5kg
	D1007215C	10kg
Seplife G25 F	D1007210F	25g
	D1007211F	100g
	D1007212F	500g
	D1007213F	1kg
	D1007214F	5kg
	D1007215F	10kg
Seplife G50 C	D1007310C	25g
	D1007311C	100g
	D1007312C	500g
	D1007313C	1kg
	D1007314C	5kg
	D1007315C	10kg
Seplife G50 F	D1007310F	25g
	D1007311F	100g
	D1007312F	500g
	D1007313F	1kg
	D1007314F	5kg
	D1007315F	10kg

Product	Ref. No.	Pack Size
Seplife G25 M	D1007210M	25g
	D1007211M	100g
	D1007212M	500g
	D1007213M	1kg
	D1007214M	5kg
	D1007215M	10kg
Seplife G25 SF	D1007210SF	25g
	D1007211SF	100g
	D1007212SF	500g
	D1007213SF	1kg
	D1007214SF	5kg
	D1007215SF	10kg
Seplife G50 M	D1007310M	25g
	D1007311M	100g
	D1007312M	500g
	D1007313M	1kg
	D1007314M	5kg
	D1007315M	10kg
Seplife G50 SF	D1007210C	25g
	D1007311SF	100g
	D1007312SF	500g
	D1007313SF	1kg
	D1007314SF	5kg
	D1007315SF	10kg

Product	Ref. No.	Pack Size
Seplife G75 M	D1007410M	25g
	D1007411M	100g
	D1007412M	500g
	D1007413M	1kg
	D1007414M	5kg
	D1007415M	10kg
Seplife G75S F	D1007410SF	25g
	D1007411SF	100g
	D1007412SF	500g
	D1007413SF	1kg
	D1007414SF	5kg
	D1007415SF	10kg
Seplife G100 M	D1007510M	25g
	D1007511M	100g
	D1007512M	500g
	D1007513M	1kg
	D1007514M	5kg
	D1007515M	10kg
Seplife G100 SF	D1007510SF	25g
	D1007511SF	100g
	D1007512SF	500g
	D1007513SF	1kg
	D1007514SF	5kg
	D1007515SF	10kg

Allyl Dextran Gel Filtration Resins

Sunresin's allyl dextran high-resolution gel filtration resins have a unique chemical structure and good physical and chemical properties. They can obtain high flow rate, high resolution and high recovery rate. These chromatography resins are widely used in the separation and purification of biological macromolecules such as enzymes, polysaccharides, nucleic acids and proteins. They are also used in the production of biological products such as γ -interferon, interleukin- II , protein A and hepatitis B vaccines.



Structure Diagram of Seplife S Series resins

• Technical Parameters of Seplife Allyl Dextran Gel Filtration Resins

Product	Particle size (μm)	Exclusion range (Globulin Da)	pH stability	Max. Flow rate, cm/h (at 0.1MPa)	Max. pressure
Seplife S-100	20-75	1000-10 \times 10 ⁴	3-11 (Operational) 2-13 (CIP)	125	The flow rate is proportional to the pressure, according to Darcy's Law
Seplife S-200	20-75	5000-25 \times 10 ⁴		150	
Seplife S-300	20-75	10000-15 \times 10 ⁵		150	

• Ordering information for Seplife Allyl Dextran Gel Filtration Resins

Product	Ref. No.	Pack size
Seplife S-100	A1007102	25ml
	A1007103	100ml
	A1007104	500ml
	A1007105	1L
	A1007106	5L
	A1007107	10L

Product	Ref. No.	Pack size
Seplife S-200	A1007202	25ml
	A1007203	100ml
	A1007204	500ml
	A1007205	1L
	A1007206	5L
	A1007207	10L

Product	Ref. No.	Pack size
Seplife S-300	A1007302	25ml
	A1007303	100ml
	A1007304	500ml
	A1007305	1L
	A1007306	5L
	A1007307	10L



Ion Exchange Chromatography Resins (IEX)

Ion Exchange Chromatography is a chromatographic method for the separation and purification of different biomolecules based on the nature and amount of charges they carry in a buffer system.

Ion Exchange Chromatography resins are porous spherical media with dissociable ionic groups (ion-exchange functional groups) bonded to their molecular matrix. According to the nature of the dissociable ion groups, they can be divided into strong acid cation exchange (SP), weak acid cation exchange (CM), strong base anion exchange (Q) and weak base anion exchange (DEAE).

• Characteristics of Ion Exchange Chromatography Resins

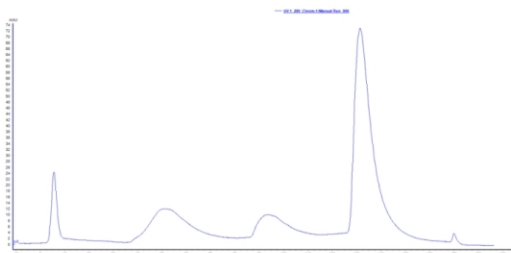
1. Resin selection and purification process design are based on the difference in the nature and strength of the charges of the targets and impurities.
2. Ion Exchange Chromatography can be used not only for the front-end capture of the target, but also for the medium and fine purification of the target. Currently it is one of the most widely used media in the field of biological separation and purification.
3. High adsorption loading and high yield.
4. Easy operation and low cost of mobile phase.
5. Good physical and chemical stability.

High Flow Rate Agarose IEX Resins

The high flow rate agarose ion exchange resins are based on the high flow rate agarose Gel Filtration chromatography resins Seplife FF, Seplife BB, Seplife HP, and through specific chemical bonding methods, the ion exchange functional ligands of different properties are firmly bonded on the agarose matrix so that they have the function of ion exchange.

• Technical parameters of High Flow Rate Agarose IEX Resins

Products	Ion Exchange Capacity (μmol/ml)	Particle Size (μm)	Max. Flow (cm/h)	Pressure Resistance (MPa)	pH Stability	Applications
SP Seplife BB	180–250 H ⁺	100–300	1800	0.3	4-13 (Operational) 3-14 (CIP)	Rapid capture and purification of charged biological macromolecules
SP Seplife FF	180–250 H ⁺	50–160	750			
SP Seplife HP	150–200 H ⁺	24–44	150			
CM Seplife BB	90–130 H ⁺	100–300	1800		4-13 (Operational) 2-14 (CIP)	
CM Seplife FF	90–130 H ⁺	45–165	750			
CM Seplife HP	90–130 H ⁺	24–44	150			
Q Seplife BB	180–250 Cl ⁻	100–300	1800		2-12 (Operational) 1-14 (CIP)	
Q Seplife FF	180–250 Cl ⁻	45–165	750			
Q Seplife HP	140–200 Cl ⁻	24–44	150			
DEAE Seplife BB	110–160 Cl ⁻	100–300	1800		2-12 (Operational) 1-14 (CIP)	
DEAE Seplife FF	110–160 Cl ⁻	45–165	750			
DEAE Seplife HP	110–160 Cl ⁻	24–44	150			



Column: XK16/20

Chromatography resins: SP Seplife FF

Mobile phase A: 20mM Phosphate buffer, pH6.8;

Mobile phase B: 20mM Phosphate buffer + 1M NaCl, pH 6.8;

Sample 1: Chymotrypsinogen

Sample 2: Cytochrome C

• Ordering information for Sunresin's high flow rate agarose IEX resins

Product	Ref. No.	Pack size
SP Seplife BB	A2036402	25ml
	A2036403	100ml
	A2036404	500ml
	A2036405	1L
	A2036406	5L
	A2036407	10L
CM Seplife BB	A2036202	25ml
	A2036203	100ml
	A2036204	500ml
	A2036205	1L
	A2036206	5L
	A2036207	10L
Q Seplife BB	A2036302	25ml
	A2036303	100ml
	A2036304	500ml
	A2036305	1L
	A2036306	5L
	A2036307	10L
DEAE Seplife BB	A2036102	25ml
	A2036103	100ml
	A2036104	500ml
	A2036105	1L
	A2036106	5L
	A2036107	10L

Product	Ref. No.	Pack size
SP Seplife FF	A2043202	25ml
	A2043203	100ml
	A2043204	500ml
	A2043205	1L
	A2043206	5L
	A2043207	10L
CM Seplife FF	A2023202	25ml
	A2023203	100ml
	A2023204	500ml
	A2023205	1L
	A2023206	5L
	A2023207	10L
Q Seplife FF	A2033202	25ml
	A2033203	100ml
	A2033204	500ml
	A2033205	1L
	A2033206	5L
	A2033207	10L
DEAE Seplife FF	A2013202	25ml
	A2013203	100ml
	A2013204	500ml
	A2013205	1L
	A2013206	5L
	A2013207	10L

Product	Ref. No.	Pack size
SP Seplife HP	A2050402	25ml
	A2050403	100ml
	A2050404	500ml
	A2050405	1L
	A2050406	5L
	A2050407	10L
CM Seplife HP	A2050202	25ml
	A2050203	100ml
	A2050204	500ml
	A2050205	1L
	A2050206	5L
	A2050207	10L
Q Seplife HP	A2050302	25ml
	A2050303	100ml
	A2050304	500ml
	A2050305	1L
	A2050306	5L
	A2050307	10L
DEAE Seplife HP	A2050102	25ml
	A2050103	100ml
	A2050104	500ml
	A2050105	1L
	A2050106	5L
	A2050107	10L

High Capacity Agarose IEX Resins

The high-capacity Seplife XL agarose ion exchange chromatography resins consist of high-strength 6% agarose connected to linear dextran molecules, which reduce the steric hindrance when they bind to the protein, thus increase the density of ion exchange ligands (DEAE/CM/Q/SP) and greatly increase the binding capacity.

- Technical parameters of High Capacity Agarose IEX Resins

Product	Ion Exchange Capacity (μmol/ml)	Particle Size (μm)	Max. Flow (cm/h)	Pressure Resistancy (MPa)	pH Stability	Application
SP Seplife XL	180–250 H ⁺	45–165	750	0.3	4-13 (Operational) 3-14 (CIP)	Ion exchange separation of proteins, nucleic acids and peptides downstream of biopharmaceuticals and bioengineering; ultra-high capacity, especially suitable for rapid capture and purification of industrial production.
CM Seplife XL	100–140 H ⁺				4-13 (Operational) 2-14 (CIP)	
Q Seplife XL	180–250 Cl ⁻				2-12 (Operational) 1-14 (CIP)	
DEAE Seplife XL	120–170 Cl ⁻				2-12 (Operational) 1-14 (CIP)	

- Ordering information for Sunresin's high capacity agarose IEX resins

Product	Ref. No.	Pack Size
DEAE Seplife XL	A2041202	25ml
	A2041203	100ml
	A2041204	500ml
	A2041205	1L
	A2041206	5L
	A2041207	10L
Q Seplife XL	A2043202	25ml
	A2043203	100ml
	A2043204	500ml
	A2043205	1L
	A2043206	5L
	A2043207	10L

Product	Ref. No.	Pack Size
CM Seplife XL	A2042202	25ml
	A2042203	100ml
	A2042204	500ml
	A2042205	1L
	A2042206	5L
	A2042207	10L
SP Seplife XL	A2044202	25ml
	A2044203	100ml
	A2044204	500ml
	A2044205	1L
	A2044206	5L
	A2044207	10L

Large Scale Agarose IEX Resins

Sunresin's Large-scale agarose ion exchange chromatography resins have faster mass transfer speed, which can significantly improve efficiency for large-scale productions. Depending on the size of the matrix, they can break down into two categories, ie. high-rigidity and high-velocity resins (Large Scale) and high-rigidity and high-resolution resins (Large Scale HP).

- Technical parameters of Large Scale Agarose IEX Resins

Products	Ion Exchange Capacity (μmol/ml)	Particle Size (μm)	Max. Flow (cm/h)	Pressure Resistance (MPa)	pH Stability	Application
SP Large Scale	110–140 H ⁺	45–165	1000	0.5	4-13 (Operational) 3-14 (CIP)	Significantly improve efficiency in large scale production.
SP Large Scale HP	130–160 H ⁺	36–44	400			
CM Large Scale	230–300 H ⁺	45–165	1000		4-13 (Operational) 2-14 (CIP)	
CM Large Scale HP	200–280 H ⁺	36–44	400			
Q Large Scale	160–220 Cl ⁻	45–165	1000		2-12 (Operational) 1-14 (CIP)	
Q Large Scale HP	150–180 Cl ⁻	36–44	400			
DEAE Large Scale	290–350 Cl ⁻	45–165	1000		2-12 (Operational) 1-14 (CIP)	
DEAE Large Scale HP	260–320 Cl ⁻	36–44	400			

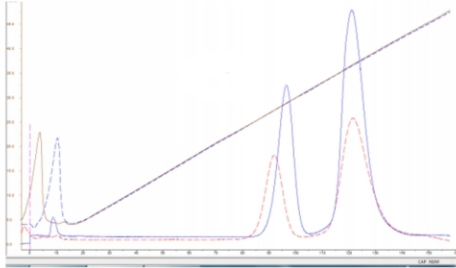
- Ordering information for Seplife Large scale IEX resins

Products	Ref. No.	Pack Size
SP Large Scale	A2046302	25ml
	A2046303	100ml
	A2046304	500ml
	A2046305	1L
	A2046306	5L
	A2046307	10L
Q Large Scale	A2036302	25ml
	A2036303	100ml
	A2036304	500ml
	A2036305	1L
	A2036306	5L
	A2036307	10L
SP Large Scale HP	A2066402	25ml
	A2066403	100ml
	A2066404	500ml
	A2066405	1L
	A2066406	5L
	A2066407	10L
Q Large Scale HP	A2066302	25ml
	A2066303	100ml
	A2066304	500ml
	A2066305	1L
	A2066306	5L
	A2066307	10L

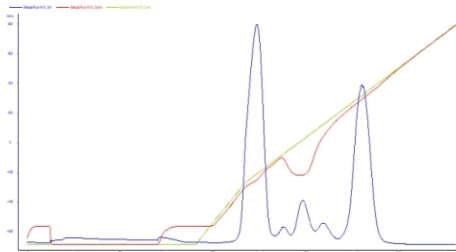
Products	Ref. No.	Pack Size
CM Large Scale	A2026302	25ml
	A2026303	100ml
	A2026304	500ml
	A2026305	1L
	A2026306	5L
	A2026307	10L
DEAE Large Scale	A2016302	25ml
	A2016303	100ml
	A2016304	500ml
	A2016305	1L
	A2016306	5L
	A2016307	10L
CM Large Scale HP	A2066202	25ml
	A2066203	100ml
	A2066204	500ml
	A2066205	1L
	A2066206	5L
	A2066207	10L
DEAE Large Scale HP	A2066102	25ml
	A2066103	100ml
	A2066104	500ml
	A2066105	1L
	A2066106	5L
	A2066107	10L

Dextran IEX Resins

Dextran matrix ion exchange chromatography resins are based on the dextran G series Gel Filtration chromatography resins Seplife G-25 and Seplife G-50, with ion-exchange functional ligands of different nature firmly bonded to the cross-linked dextran molecular backbone, making them ion-exchange capable chromatography resins.



Column: XK16/20 CM25
Sample 1: Quinine Sulfate
Sample 2: Lysozyme



Chromatographic conditions:

Mobile phase A: 20mmol/L Phosphate buffer
Mobile phase B: 20mmol/L Phosphate buffer + 1mol/L NaCl
Elution method: Gradient elution

• Technical Parameters of Dextran Ion Exchange Chromatography Resins

Product	Ion Exchange Capacity (mmol/g)	Particle Size (μm)	Binding Capacity (ng/ml Chromatography Medium)	Max. Flow (cm/h)	Pressure Resistance (Mpa)	pH Stability	Applications
DEAE Seplife A25	3.0-4.0	40-120	140 (α -Lactalbumin)	120	0.24	2-13 (CIP) 2-9 (Operational)	Purification of Low molecular weight proteins, peptides, nucleotides and macromolecules.
DEAE Seplife A50	3.0-4.0	40-120	110 (albumin)	60	0.1	2-12 (CIP) 2-9 (Operational)	
CM Seplife C25	4.0-5.0	40-120	190 (Ribonucleotide)	120	0.24	2-13 (CIP) 6-10 (Operational)	
CM Seplife C50	4.0-5.0	40-120	120 (Ribonucleotide)	100	0.1	2-12 (CIP) 6-10 (Operational)	
Q Seplife A25	2.5-3.5	40-120	110 (α -Lactalbumin)	100	0.24	2-13 (CIP) 2-12 (Operational)	
Q Seplife A50	2.5-3.5	40-120	80 (albumin)	60	0.1	2-12 (CIP) 2-12 (Operational)	
SP Seplife C25	2.0-2.5	40-120	230 (Ribonucleotide)	100	0.24	2-13 (CIP) 4-13 (Operational)	
SP Seplife C50	2.0-2.5	40-120	100 (Ribonucleotide)	100	0.1	2-12 (CIP) 4-13 (Operational)	

- Ordering information for dextran ion exchange chromatography resins

Product	Ref. No.	Pack Size
DEAE Seplife A25	D2017210	25g
	D2017211	100g
	D2017212	500g
	D2017213	1kg
	D2017214	5kg
	D2017215	10kg
DEAE Seplife A50	D2017310	25g
	D2017311	100g
	D2017312	500g
	D2017313	1kg
	D2017314	5kg
	D2017315	10kg
CM Seplife C25	D2027210	25g
	D2027211	100g
	D2027212	500g
	D2027213	1kg
	D2027214	5kg
	D2027215	10kg
CM Seplife C50	D2017310	25g
	D2017310	100g
	D2017310	500g
	D2017310	1kg
	D2017310	5kg
	D2017310	10kg

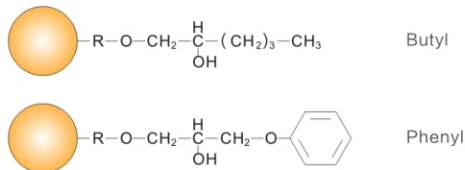
Product	Ref. No.	Pack Size
Q Seplife A25	D2037210	25g
	D2037211	100g
	D2037212	500g
	D2037213	1kg
	D2037214	5kg
	D2037215	10kg
Q Seplife A50	D2037310	25g
	D2037311	100g
	D2037312	500g
	D2037313	1kg
	D2037314	5kg
	D2037315	10kg
SP Seplife C25	D2047210	25g
	D2047211	100g
	D2047212	500g
	D2047213	1kg
	D2047214	5kg
	D2047215	10kg
SP Seplife C50	D2047310	25g
	D2047310	100g
	D2047310	500g
	D2047310	1kg
	D2047310	5kg
	D2047310	10kg



Hydrophobic Interaction Chromatography Resins (HIC)

Hydrophobic Interaction Chromatography (HIC) is a method of protein separation based on the difference in the interaction between different proteins and hydrophobic surfaces. In general, the higher the ionic strength (salt concentration), the stronger the hydrophobic bond formed with the substance. Factors that influence hydrophobic interactions include salt concentration, temperature, pH, surface activators and organic solvents.

Sunresin proposes two types of commonly used HIC resins. Butyl and Phenyl groups are covalently bonded to Seplife FF-based matrix via a short linker. The structure is shown in the following diagram.

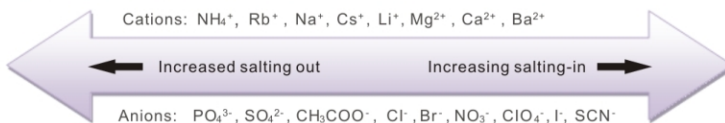


Basic operations of Hydrophobic interactions

1. Selection of the chromatographic column

The height of the column bed is usually 5-15cm. In large scale production, the height can be kept unchanged and the diameter of the column can be increased.

2. Selection of the buffer conditions



3. Selection of buffer

- When pH increases, hydrophobicity decreases accordingly
- When pH increases, hydrophilicity of the protein increases due to an increase in the number of charged groups being neutralized at increasing pH
- Proteins that do not bind to hydrophobic resins at neutral pH conditions can bind at acidic pH conditions

4. Elution of proteins

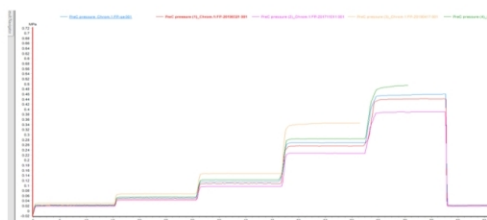
- Low concentrations of water-soluble ethanol, detergents and salts with a salting-in effect, destroy the structure of water and reduce the surface tension, thus weakening hydrophobic interactions.
- The non-polar region of the ethanol or detergent competes with the bound protein for the hydrophobic ligand, thereby replacing the protein.

5. Regeneration, cleaning and storage of the column

- When slightly contaminated, wash with distilled water.
- For tightly bound contaminants, wash with 6 mol/L urea or 6 mol/L guanidine HCL and start buffer or storage buffer
- NaOH can be used to dissolve denatured and precipitated proteins and lipids.
- Unused resins should be stored in closed containers at 4-25°C.
- Used resins should be stored in a solution containing an appropriate bacteriostatic agent at 4-8°C. Avoid freezing.

- Technical Parameters of Seplife Hydrophobic Chromatography Resins

Product	Matrix	Particle Size (µm)	Adsorption Capacity (mg/ml)	Max. Flow (cm/h)	Pressure Resistance (Mpa)	pH Stability
Phenyl Seplife FF (LS)	Seplife 6FF	45-165	>33 (HSA)	750	0.3	3-13 (Operational) 2-14 (CIP)
Phenyl Seplife FF(HS)		45-165	>40 (HSA)	750	0.3	3-13 (Operational) 2-14 (CIP)
Phenyl Seplife HP	Seplife HP	24-44	>24 (HSA)	150	0.3	3-13 (Operational) 2-14 (CIP)
Butyl Seplife 4FF	Seplife 4FF	45-165	>26 (HSA)	420	0.3	3-13 (Operational) 2-14 (CIP)
Butyl-S Seplife FF		45-165	>26 (HSA)	420	0.3	3-13 (Operational) 2-14 (CIP)
Butyl Seplife 4B	Seplife 4B	45-165	>26 (HSA)	120	0.1	3-13 (Operational) 2-14 (CIP)
Butyl Seplife HP	Seplife 6FF	24-44	>35 (HSA)	150	0.3	3-13 (Operational) 2-14 (CIP)
Phenyl Large Scale	Large Scale	75 (D50V)	>27 (HSA)	1000	0.5	3-13 (Operational) 2-14 (CIP)
Butyl Large Scale			>27 (HSA)	1000	0.5	3-13 (Operational) 2-14 (CIP)
Phenyl Large Scale HP	Large Scale HP	40 (D50V)	>19 (HSA)	400	0.5	3-13 (Operational) 2-14 (CIP)
Butyl Large Scale HP			>37 (HSA)	400	0.5	3-13 (Operational) 2-14 (CIP)



Product Pressure Curve

Column: XK16*20

Mobile phase: 0.1mol/L NaCl

• Ordering information for Hydrophobic Interaction Chromatography resins

Product	Ref. No.	Pack Size
Phenyl Seplife FF (LS)	A3013202	25ml
	A3013203	100ml
	A3013204	500ml
	A3013205	1L
	A3013206	5L
	A3013207	10L
Phenyl Seplife FF (HS)	A3023202	25ml
	A3023203	100ml
	A3023204	500ml
	A3023205	1L
	A3023206	5L
	A3023207	10L
Phenyl Seplife HP	A3015202	25ml
	A3015203	100ml
	A3015204	500ml
	A3015205	1L
	A3015206	5L
	A3015207	10L
Phenyl Large Scale	A3063202	25ml
	A3063203	100ml
	A3063204	500ml
	A3063205	1L
	A3063206	5L
	A3063207	10L
Phenyl Large Scale HP	A3615202	25ml
	A3653203	100ml
	A3653204	500ml
	A3653205	1L
	A3653206	5L
	A3653207	10L

Product	Ref. No.	Pack Size
Butyl Seplife 4FF	A3033102	25ml
	A3033103	100ml
	A3033104	500ml
	A3033105	1L
	A3033106	5L
	A3033107	10L
Butyl-S Seplife FF	A3043102	25ml
	A3043103	100ml
	A3043104	500ml
	A3043105	1L
	A3043106	5L
	A3043107	10L
Butyl Seplife 4B	A3051102	25ml
	A3051103	100ml
	A3051104	500ml
	A3051105	1L
	A3051106	5L
	A3051107	10L
Butyl Large Scale	A3063302	25ml
	A3063303	100ml
	A3063304	500ml
	A3063305	1L
	A3063306	5L
	A3063307	10L
Butyl Large Scale HP	A3653302	25ml
	A3653303	100ml
	A3653304	500ml
	A3653305	1L
	A3653306	5L
	A3653307	10L



Affinity Chromatography

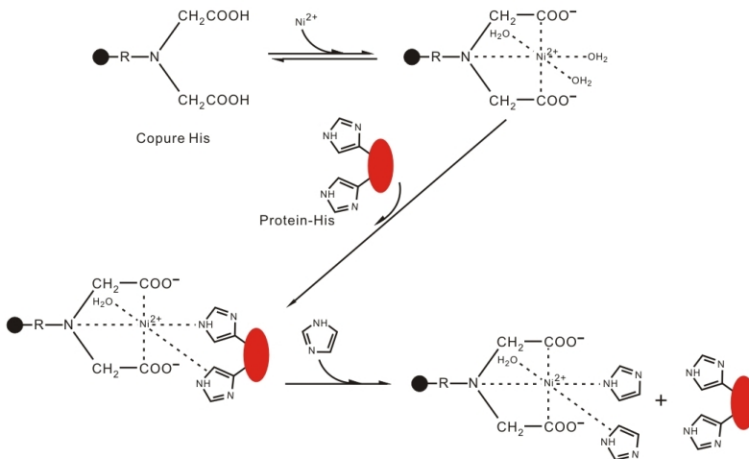
Affinity chromatography is a chromatographic method for separating proteins based on specific interactions between biomolecules, such as enzymes and substrates, receptors and ligands, antibodies and antigens. This interaction is specific and reversible, and protein purification is achieved through this reversible binding and separation.

• Characteristics of Affinity Chromatography

- High selectivity and efficiency, high purification fold based on the specific affinity between ligand and macromolecule to be separated.
- Ability to complete separation that is otherwise difficult to complete in one step, such as denatured and undenatured proteins with different functions.
- Typically over 90% purity can be achieved in one step.
- Fast and easy operation.

Metal Chelate Affinity Resins

Ni Seplife FF (IDA) is an affinity chromatography resin formed by bonding iminodiethyl to a fast flow 6B agarose matrix and then chelating metal ions ($\text{Cu}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+}$). It uses the affinity adsorption of amino acid residues such as histidine and metal ions in the sample components for separation and purification.



This product is a white spherical bead when it is not chelated with metal ions. It shows different colors if chelated with different metal ions.

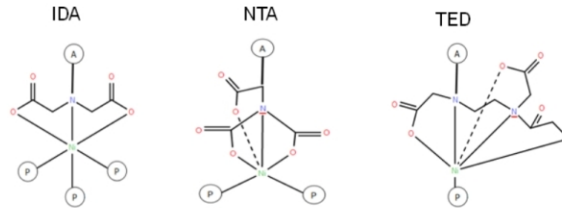
Ni Seplife FF (NTA) is an affinity resin formed by bonding aminotriacetic acid to fast flow rate 6B agarose matrix. It can bond metal ions at the trivalent level, making the chelated metal ions more stable and resistant to higher reducing agents. It is physically and chemically stable and has the advantage of good specificity and fast flow rates.

Ni Seplife FF (TED) is an affinity resin formed by bonding N,N,N'-tris(carboxymethyl) ethylene diammonium to fast flow rate 6B agarose matrix. It can bond metal ions at the pentavalent level, making the chelated metal ions more stable and resistant to 10 mM of reducing agents, 100mM of EDTA and 1M of NaOH. For samples containing chelating agents and reducing agents such as EDTA, the sample can be directly loaded without replacing the buffer. Also, there is no need to remove metal ions during maintenance. It can be directly cleaned and disinfected with 1M sodium hydroxide, which is convenient for purification of His tag proteins on a large scale.

• Technical Parameters of Metal Chelate Affinity Resins

Product	Binding Capacity (1ml media)	Particle Size (µm)	Max. Flowrate (cm/h)	Pressure Resistance (Mpa)	pH Stability	Application
Ni Seplife FF(IDA)	45mg His- tagged proteins	45-165	750	0.3	2-14 (CIP) 3-12 (Operational)	Large scale purification of His- tagged proteins
Ni Seplife FF(NTA)	40mg His- tagged proteins	45-165	750			
Ni Seplife HP (IDA)	40mg His- tagged proteins	25-45	150			
Ni Seplife FF(TED)	20mg His- tagged proteins	45-165	750			Resistant to 100 mM of EDTA and 10mM of DTT. No need to remove nichel. Clean thouroughly with 1M of NaOH.
IMAC Seplife FF(IDA)	25 µ mol Cu ²⁺	45-165	750			Chelate metal ions, purify His tag protein
IMAC Seplife FF(NTA)	20 µ mol Cu ²⁺	45-165	750			

Ni²⁺ affinity resin is the most widely used metal chelate chromatography resin in purification experiments. Depending on the binding group, it can also be divided into Ni-IDA, Ni-NTA and Ni-TED. Ni²⁺ has six chelating valencies, of which Ni-IDA chelates the trivalent, Ni-NTA the tetravalent and Ni-TED the pentavalent. The Ni-IDA has the highest binding capacity, while Ni-TED has the best resistance and stability.



• Ordering information for Ni Seplife affinity resins

Product	Ref. No.	Pack Size
Ni Seplife FF(IDA)	A4013202	25ml
	A4013203	100ml
	A4013204	500ml
	A4013205	1L
	A4013206	5L
	A4013207	10L
Ni Seplife HP(IDA)	A4015202	25ml
	A4015203	100ml
	A4015204	500ml
	A4015205	1L
	A4015206	5L
	A4015207	10L

Product	Ref. No.	Pack Size
Ni Seplife FF(NTA)	A4023202	25ml
	A4023203	100ml
	A4023204	500ml
	A4023205	1L
	A4023206	5L
	A4023207	10L
IMAC Seplife FF(IDA)	A4003012	25ml
	A4003013	100ml
	A4003014	500ml
	A4003015	1L
	A4003016	5L
	A4003017	10L

Product	Ref. No.	Pack Size
Ni Seplife FF(TED)	A4153202	25ml
	A4153203	100ml
	A4153204	500ml
	A4153205	1L
	A4153206	5L
	A4153207	10L
IMAC Seplife FF(NTA)	A4003022	25ml
	A4003023	100ml
	A4003024	500ml
	A4003025	1L
	A4003026	5L
	A4003027	10L

Affinity Resins for Purification of GST- Tagged Proteins

The Glutathione ligand is coupled to a 4% high-flow agarose chromatography resin through a 12-atom linking arm. This coupling allows the medium to have a stronger binding ability to GST-tagged proteins and other glutathione binding proteins. Glutathione SepLife 4FF's high protein binding capacity and high flow rate facilitate large-scale production.

• Technical Parameters for Affinity Resins for GST- Tagged Protein Purification

Product	Matrix	Particle Size (µm)	Max Flowrate (cm/h)	Binding Capacity (mg/ml)	pH Stability
Glutathione SepLife 4FF	SepLife 4FF	45–165	420	10 (GST fusion protein)	22-14 (CIP) 3-12 (Operation)

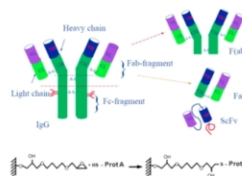
• Ordering information for affinity resins for GST- tagged protein purification

Product	Ref. No.	Pack Size
Glutathione SepLife 4FF	A4053102	25ml
	A4053103	100ml
	A4053104	500ml
	A4053105	1L
	A4053106	5L
	A4053107	10L

Affinity Resins for Antibody Purification

The r-protein A SepLife 4FF specifically binds to the Fc region of antibodies and is used for the isolation and purification of monoclonal or polyclonal antibodies. In one step, high purity antibodies can be obtained from samples such as ascites, serum or culture solution.

The r-protein G SepLife 4FF can be used for the isolation and purification of antibodies of different genera or Fc fragments of antibodies in cell cultures or cell extracts, with multi-site activated coupling to reduce the leakage of ligands. It is used for laboratory-scale and industry-scale purification of IgG monoclonal antibodies and can rapidly process large sample volumes.



• Technical Parameters of SepLife Affinity Resins for Antibody Purification

Product	Particle Size (µm)	Binding Capacity (1ml medium)	Max. Flowrate (cm/h)	Pressure Resistance (MPa)	pH Stability
rProtein A SepLife Suni	45–165	50mg Human IgG	420	0.3	3-9 (Operational) 2-10 (CIP)
rProtein A SepLife Suno		70mg Human IgG	420	0.3	
rProtein G SepLife 4FF		30mg Human IgG	420	0.3	

• Ordering information for SepLife affinity resins for antibody purification

Product	Ref. No.	Pack Size
rProtein A SepLife Suni	A4073101	5ml
	A4073102	25ml
	A4073103	100ml
	A4073104	500ml
	A4073105	1L
	A4073106	5L
	A4073107	10L

Product	Ref. No.	Pack Size
rProtein A SepLife Suno	A4093101	5ml
	A4093102	25ml
	A4093103	100ml
	A4093104	500ml
	A4093105	1L
	A4093106	5L
	A4093107	10L

Product	Ref. No.	Pack Size
rProtein G SepLife 4FF	A4083101	5ml
	A4083102	25ml
	A4083103	100ml
	A4083104	500ml
	A4083105	1L
	A4083106	5L
	A4083107	10L

Affinity Resins for Plasmid DNA Purification

Plasmid Seplife LX is a new type of highly cross-linked thiophilic aromatic agarose chromatography resin independently developed by Sunresin. It uses the sulfophilic adsorption of the ligand and is suitable for the purification of closed-loop supercoiled plasmid DNA. It is widely applied to the applications of gene therapy and DNA vaccine production. This chromatography resin has the characteristics of high flow rate, low back pressure, low non-specific adsorption, good hydrophilicity, chemical stability and mechanical properties, which is convenient for scale-up and can shorten production time and improve production efficiency.

• Technical Parameters of Plasmid Seplife LX

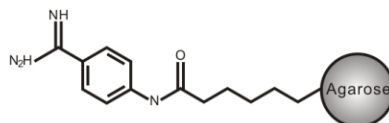
Product	Ligand Concentration (μmol/mL)	Particle Size (μm)	Binding Capacity (mg/ml)	Max. Flowrate (cm/h)	Pressure Resistance (Mpa)	pH Stability	Chemical Stability
Glutathione Seplife 4FF	27–50 (Mercaptopyrindine)	36–44	≥3	220	0.3	3–13 (Operational) 2–14 (CIP)	Stable in the following liquids: all commonly used aqueous buffers; 1mol/L sodium hydroxide; 70% ethanol; 40% isopropanol; 1M acetic acid

• Ordering information for Plasmid Seplife LX

Product	Ref. No.	Pack Size
Seplife Plasmid LX	A4207101	25ml
	A4207102	100ml
	A4207103	500ml
	A4207104	1L
	A4207105	5L
	A4207106	10L

Affinity Resins for Serine Protease Purification

Benzamides are broad-spectrum inhibitors of serine proteases, which can be coupled to fast-flow agarose gel filtration chromatography resins to purify serine proteases such as trypsin, thrombin, urokinase, kallikrein, prekallikrein, etc, in one step from various sources of samples.



• Technical Parameters of Benzamide seplife 4FF

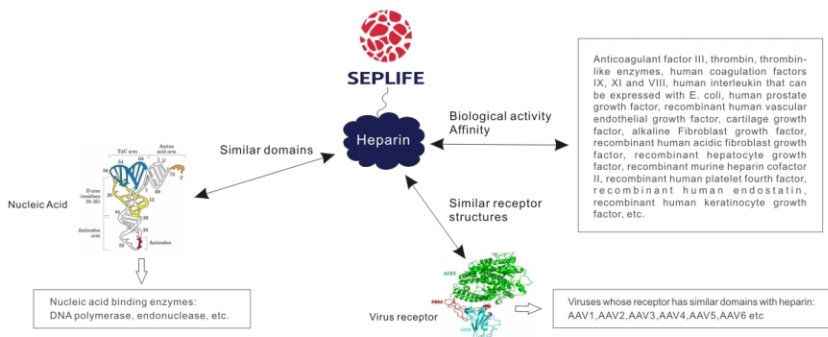
Product	Matrix	Particle Size (μm)	Ligand Concentration (μmol/ml)	Binding Capacity (mg/ml)	Max. Flowrate (cm/h)	pH Stability
Benzamide Seplife 4FF	Seplife 4FF	45-165	12 (P-aminobenzamide)	>35 (Trypsin)	420	3-10(Operational) 2-11(CIP)

• Ordering information for Benzamide seplife 4FF

Product	Ref. No.	Pack Size
Benzamide Seplife 4FF	A4043102	25ml
	A4043103	100ml
	A4043104	500ml
	A4043105	1L
	A4043106	5L
	A4043107	10L

Heparin Affinity Resin

Heparin is an acidic polysaccharide containing sulphate esters that bind to biomolecules such as anticoagulant factor III, coagulation factors, lipoproteins, interferons, nucleic acid binding proteins, restriction endonucleases, thrombin and thrombin-like enzymes, and couple them to activated cross-linked agarose media, used for the purification of these substances.



• Technical Parameters of Seplife Heparin Affinity Resins

Product	Matrix	Particle Size (µm)	Ligand Concentration (mg/ml)	Binding Capacity (/1ml resin)	Max. Flowrate cm/h	pH Stability	Storage
Heparin Seplife CL-6B	Seplife CL-6B	45-165	>5	>3mg (Anticoagulant factor III)	240	4-10 (Operational) 3-13 (CIP)	4-8°C, 20% ethanol solution containing 0.05mol/L NaAc
Heparin Seplife FF	Seplife FF	45-165	>5	>3mg (Anticoagulant factor III)	750		

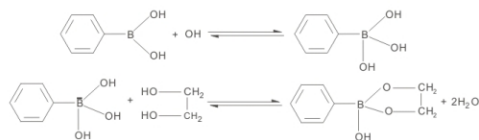
• Ordering information for Seplife Heparin affinity resins

Product	Ref. No.	Pack Size
Heparin Seplife CL 6B	A4032202	25ml
	A4032203	100ml
	A4032204	500ml
	A4032205	1L
	A4032206	5L
	A4032207	10L

Product	Ref. No.	Pack Size
Heparin Seplife FF	A4033202	25ml
	A4033203	100ml
	A4033204	500ml
	A4033205	1L
	A4033206	5L
	A4033207	10L

Boric Acid Affinity Resins

Boric acid is able to bind to 1,2 cis-diols, so boric acid agarose resins can be used for the purification of sugars, nucleosides, nucleic acids, peptides and enzymes, and are particularly suitable for the purification of glycoproteins. This product couples boric acid compounds to activated agarose 4FF matrix and can be used for the purification of these substances.



• Technical Parameters of Seplife Boric Acid Affinity Resins

Product	Matrix	Particle Size (μm)	Ligand Concentration (μmol/ml)	Binding Capacity (/1ml resin)	Max. Flowrate (cm/h)	pH Stability	Storage
Boricacid Seplife 4FF	Seplife 4FF	45-165	20	10 mg ConA	420	3-10 (Operational) 2-13 (CIP)	4-8°C

• Ordering information for Seplife Boric acid affinity resins

Product	Ref. No.	Pack Size
Boricacid Seplife 4FF	A4063102	25ml
	A4063103	100ml
	A4063104	500ml
	A4063105	1L
	A4063106	5L
	A4063107	10L

Blue Dye Affinity Resins (Blue Seplife FF)

Blue Seplife FF is an affinity chromatography resin that couples Cibacron Blue ligand to high flow rate agarose matrix, which has a high chemical stability. Its binding to the target protein is mainly the result of a combination of electron pairs provided by the ligand and hydrophobic interactions and is suitable for the purification of albumin, interferon, coagulation factors and various enzymes requiring NAD⁺ and NADP⁺.

• Technical Parameters of Blue Seplife FF

Product	Matrix	Particle Size (μm)	Ligand Concentration (μmol/ml)	Max. Flowrate (cm/h)	pH Stability	Storage
Blue Seplife FF	Seplife 6FF	45-165	7(Cibacron Blue)	750	3-13 (CIP) 4-12 (Operational)	2-8°C, 0.1mol/L Potassium dihydrogen phosphate, 20% ethanol solution at pH8.0

• Ordering information for Blue Seplife FF

Product	Ref. No.	Pack Size
Blue Seplife FF	A4193202	25ml
	A4193203	100ml
	A4193204	500ml
	A4193205	1L
	A4193206	5L
	A4193207	10L

Red Dye Affinity Resins (Red Seplife FF)

Red Seplife FF is an affinity resin that covalently binds Reactive Red 120 to high flow rate agarose matrix. The Reactive Red 120 is a polycyclic dye with some structural similarities to naturally occurring NADP⁺ that binds strongly and specifically to a wide range of proteins, such as lactate dehydrogenase. The proteins that bind specifically to the red dye do so because they require a nucleotide co-factor. Other proteins, such as albumin, lipoproteins, coagulation factors and interferons, bind to the red dye in a non-specific manner, such as electrostatic interactions of aromatic ring anion ligands or hydrophobic bond interactions.

• Technical Parameters of Red Seplife FF

Product	Matrix	Particle Size (µm)	Ligand Concentration (µmol/ml)	Max. Flowrate (cm/h)	pH Stability	Storage
Red Seplife FF	Seplife 6FF	45-165	2(Reactive Red 120)	750	3-13 (CIP) 4-12 (Operational)	2-8°C, 20% ethanol solution containing 1 mol/L NaCl

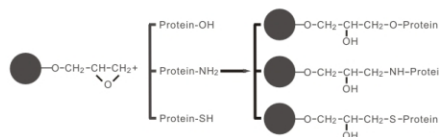
• Ordering information for Red Seplife FF

Product	Ref. No.	Pack Size
Red Seplife FF	A4203202	25ml
	A4203203	100ml
	A4203204	500ml
	A4203205	1L
	A4203206	5L
	A4193207	10L

Pre-activated Chromatography Resins

Epoxy Activated Seplife 4FF is a reactive intermediate formed by bonding epoxy groups to the agarose 4FF matrix. It can be directly coupled to ligands to prepare various separation media for the purification of biomolecules.

CNBr-Activated Seplife 4FF is an activated intermediate that reacts with the hydroxyl group on top of the agarose to form an activated cyanate group. Proteins, peptides and amino acids can be coupled to hydrogen bromide activated 4FF agarose resins and are widely used.



• Technical Parameters of Seplife Pre-activated Chromatography Resins

Product	Matrix	Particle Size (µm)	Ligand Concentration (µmol/ml)	Max. Flowrate (cm/h)	Pressure Resistance (MPa)	pH Stability	Storage
Epoxy Activated Seplife 4FF	Seplife 4FF	45-165	20-40 (Epoxy-based)	420	0.3	2-14	Freeze-dried samples: sealed and stored below 8°C Swollen resins: stored in 20% ethanol at 4°C-8°C Avoid contact with oxidants
CNBr Activated Seplife 4FF	Seplife 4FF	45-165	20-60 (α-Pancreatic prolectinogen)	420	0.3	2-11	

• Ordering information for Seplife Pre-activated chromatography resins

Product	Ref. No.	Pack Size
Epoxy Activated Seplife 4FF	A4101108	5g
	A4101109	10g
	A4101110	25g
	A4101111	100g
	A4101112	500g
	A4101113	1kg
	A4101114	5kg
	A4101115	10kg

Product	Ref. No.	Pack Size
CNBr Activated Seplife 4FF	A4031008	5g
	A4031009	10g
	A4031010	25g
	A4031011	100g
	A4031012	500g
	A4031013	1kg
	A4031014	5kg
	A4031015	10kg



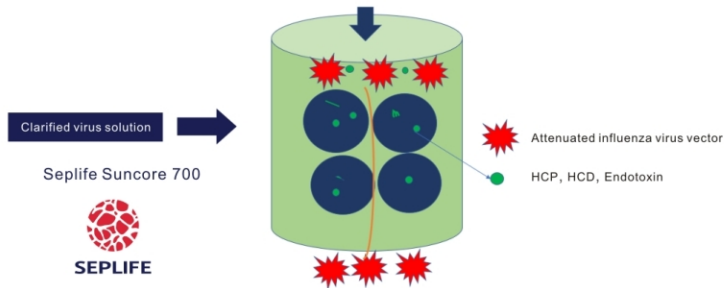
Multimodal Chromatography Resins (MM)

Seplife Suncore 700 Multimodal Resins

Seplite Suncore 700 is a polyacrylate agarose microsphere with octylamine functional groups bonded to the core. The outside of the polyacrylate agarose microspheres is an inert shell, and the size exclusion limit is 700KDa.

Under the condition of high conductivity, the target substance larger than 700KDa flows through the gap between the microspheres, and the target substance is purified by the principle of gel filtration;

When the impurities less than 700KDa enter the inner core of the microspheres, they are adsorbed and combined with the octylamine functional groups inside the microspheres. Through multi-mode actions such as ion exchange and hydrophobic interaction, impurities such as host proteins in the target are removed.



• Technical Parameters for Seplite Suncore 700

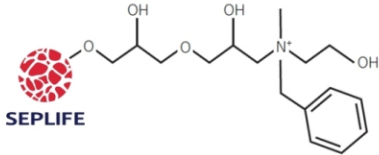
Product	Ligand	Ion Content (μmol/ml)	Particle Size d _{50v} (μm)	Max. Flowrate (cm/h)	Pressure Resistance (MPa)	pH Stability	Chemical Stability
Seplite Suncore 700	Octylamine	40-85 Cl ⁻	85	500	0.3	3-13 (Operational) 2-14 (CIP)	Stable at all commonly used aqueous buffers; 1mol/L sodium hydroxide; 70% ethanol; 30% isopropanol; 6M guanidine hydrochloride

• Ordering information for Seplite Suncore 700

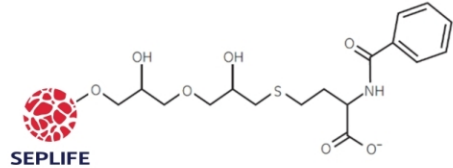
Product	Ref. No.	Pack Size
Seplite Suncore 700	A1008101	25ml
	A1008102	100ml
	A1008103	500ml
	A1008104	1L
	A1008105	5L
	A1008106	10L

MA/MMC Seplife Multimodal Resins

MA Seplife is a Multimodal chromatography resin that has anion exchange, hydrophobic interaction and hydrogen bonding capabilities at the same time, specifically for the medium/fine purification of monoclonal antibodies. (As shown on the left)



MMC Seplife is a multimodal resin that has cation exchange, hydrophobic interaction and hydrogen bonding capacities at the same time. It can bind to proteins under high salt conditions. Samples can be loaded directly without dilution. (As shown on the left)



• Technical Parameters of MA/MMC Multimodal Chromatography Resins

Product	Matrix	Functional Group	Particle Size (d _{50v} , μm)	Ion Content (μmol/ml)	Binding Capacity (mg/ml)	Max. Flowrate (cm/h)	Pressure Resistance (MPa)	pH Stability
MMC Large Scale	Large Scale	Multimode Weak cation	75	0.07-0.09	≥45 (BSA)	1000	0.5	3-12 (Operational) 3-14 (CIP)
MA Large Scale		Multimode Strong anion		0.09-0.12	50-90 (Mab)			3-12 (Operational) 2-14 (CIP)
MMC Large Scale HP	Large Scale HP	Multimode Weak cation	40	0.06-0.08	60-90 (Mab)	400	0.5	3-12 (Operational) 3-14 (CIP)
MA Large Scale HP		Multimode Strong anion		0.08-0.11	45-85 (Mab)			3-12 (Operational) 2-14 (CIP)

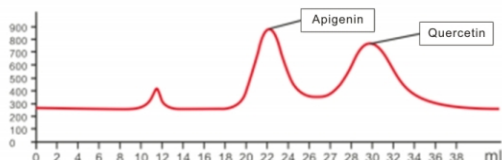
• Ordering information for MA/MMC Seplife resins

Product	Ref. No.	Pack Size
MMC Large Scale	A5016302	25ml
	A5016303	100ml
	A5016304	500ml
	A5016305	1L
	A5016306	5L
	A5016307	10L
MMC Large Scale HP	A5050102	25ml
	A5050103	100ml
	A5050104	500ml
	A5050105	1L
	A5050106	5L
	A5050107	10L

Product	Ref. No.	Pack Size
MA Large Scale	A5026302	25ml
	A5026303	100ml
	A5026304	500ml
	A5026305	1L
	A5026306	5L
	A5026307	10L
MA Large Scale HP	A5050202	25ml
	A5050203	100ml
	A5050204	500ml
	A5050205	1L
	A5050206	5L
	A5050207	10L

Seplife LH-20 Multimodal Resins

Seplife LH-20 chromatography resin uses dextran G-25 as the matrix and is obtained through hydroxypropylation. It combines gel filtration, partition chromatography and adsorption chromatography. Seplife LH-20 chromatography resin is suitable for the purification of lipophilic small molecule compounds. It can separate organics with very similar structural properties. It can be used for preliminary purification steps or final purification steps, such as monomers. Purification or separation of diastereoisomers is especially suitable for the separation of physiologically active ingredients in herbal medicines, such as polyphenolic flavonoids, cholesterol, fatty acids, hormones, vitamins, etc.



Effect of LH-20 on the separation of apigenin and quercetin

Column: XK16/70

Sample:

1. Apigenin
2. Quercetin

• Technical Parameters of Seplife LH-20 Chromatography Resins

Product	Particle Size (dry μm)	Swelling factor ml/g	Exclusion Range (globulin Da)	Max. Flowrate (cm/h)	pH Stability	Pressure Resistance (MPa)
Seplife LH-20	18-111	4-6	1000-5000	480	3-10 (Operational) 2-13 (CIP)	The flow rate is proportional to the pressure, according to Darcy's Law.

• Ordering information for Seplife LH-20 chromatography resins

Product	Ref. No.	Pack Size
Seplife LH-20	D6007610	25g
	D6007611	100g
	D6007612	500g
	D6007613	1kg
	D6007614	5kg
	D6007615	10kg



Seplife HM Chromatography Resins

With the development of the bio-pharma industry, the requirements for the efficiency of downstream purification process are gradually increasing. In practice, separation media with high pressure tolerance and high flow rates are more advantageous. Therefore, in recent years, rigid structured chromatography resins have been increasingly used in the bio-pharma field and have shown superior features to traditional agarose and dextran-based media in the purification of certain products.

Seplife HM chromatography resins are based on a unique rigid matrix with hydrophilic modification of the surface and group bonding, resulting in good hydrophilicity, high loading capacity, long service life and high flow rate. Compared to traditional chromatography resins, they can greatly improve the production efficiency of downstream purification process, reduce the cost and create good economic benefits.

• Ordering information for Seplife LH-20 chromatography resins

Purification targets	Antibodies	Recombinant proteins	Blood products	Vaccines (human and animal)	Nucleic acids	Peptides	Biological small molecules	Natural products
Chromatography methods	Affinity Ion exchange	Ion exchange Affinity Gel filtration Reverse phase	Ion exchange Affinity Gel filtration	Ion exchange Gel filtration Microcarriers (cell culture) Multifunctional composite model	Affinity Ion exchange Reverse phase	Ion exchange Reverse phase	Ion exchange Reverse phase	Reverse phase

• Features of Seplife HM chromatography resins

1. High dynamic loading capacity
2. High flow rate purification for increased productivity
3. Good stability and easy in-line cleaning
4. Uniform particle size with high resolution

• Technical Parameters of Seplife HM Chromatography Resins

Item	Parameters	Remarks
Skeletal matrix	Polymethyl methacrylate	
Particle size (µm)	S: 25-45; M: 45-90; C: 90-120; EC:100-300	
Pore size (Å)	800-1200	
Pressure resistant flow rate (cm/h)	S:400-600;M:800-1200;C:1000-1200	22mm×20cm,3bar, Compression factor ≤ 1.05
Maximum pressure resistance (bar)	8	
Storage	20% Ethanol solution	
Regeneration	0.5-2M NaCl solution	
Cleaning	0.1-0.5M NaOH solution (or 50% ethanol)	

Seplife HM Ion Exchange Chromatography Resins

Seplife HM Ion Exchange Chromatography Resins are produced by using a proprietary synthesis technique of polymethacrylate as the backbone of the microspheres, followed by surface modification of the hydrophilic base spheres and finally bonding of different ion exchange functional groups. They have the advantages of high strength, high flow rate and high recovery rate and are suitable for mass production.

• Classification of ion exchange groups

Functional group type	Structural formula	Abbreviations
Weak acid cation	-O-CH ₂ -COO-	CM
Strong acid cation	-O-(CH ₂) ₃ -SO ₃ -	SP
Weak base anion	-O-(CH ₂) ₂ -NH ⁺ -(CH ₂ CH ₃) ₂	DEAE
Weak base anion	-CH ₂ -NH ⁺ -(CH ₂ CH ₃) ₂	DEAE
Strong base anion	-O-(CH ₂) ₂ -N ⁺ -(CH ₃) ₃	Q
Strong base anion	-CH ₂ -N ⁺ -(CH ₃) ₂ -CH ₂ -CH ₂ -OH	DMAE
Strong base anion	-O-N ⁺ -(C ₂ H ₅) ₃	QAE
Composite mode	-CH ₂ -N ⁺ -CH ₃ (-CH ₂ -CH ₂ -OH, -C ₆ H ₅)	

• Product technical parameters

Name	Particle size	Pore size	Ion Exchange capacity meq/ml	Dynamic load capacity mg/ml	Abbreviations
LX-Seplife Q650	S,M,C	1000	≧ 0.24	90	Strongly basic anions
LX-Seplife Q504	S,M,C	1000	≧ 0.3	100	
LX-Seplife Q400	S,M,C	400	≧ 0.2	60	
Mixed mode	M	1000	≧ 0.1	90	
LX-Seplife DEAE504	S,M,C	1000	≧ 0.4	100	Weakly alkaline anions
LX-Seplife SP504	S,M,C	1000	≧ 0.3	110	Strong acid cations
LX-Seplife CM504	S,M,C	1000	≧ 0.45	90	Weak acid cations

Seplife HM Metal Chelate Chromatography Resins

Seplife HM metal chelate affinity chromatography resins are based on poly (methyl methacrylate) with a hydrophilic modified surface with good physicochemical stability, biocompatibility and solvent compatibility for the separation and purification of biological macromolecules. Customers can chelate different transition metal ions (Ni^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Fe^{3+} , etc.) as required. Seplife HM55IDA and Seplife HM55NTA are chelated with the metal ion Ni^{2+} and can be used directly.

- Product performance indicators

Product name	Particle size (um)	Pore size (Å)	Max. pressure resistance(bar)	Linear flow rate (cm/h)	Dynamic load capacity (HIS- tagged protein, mg/ml)	pH stability range
Ni Seplife IDA55 S	35-50	1000	8	100-800	≥ 30	2-12
Ni Seplife IDA55 M	45-90					
Ni Seplife NTA55 S	35-50	1000	8	100-800	≥ 20	2-12
Ni Seplife NTA55 M	45-90					

- Ordering information for Ni Seplife IDA55 and Ni Seplife NTA55 chromatography resins

Product Name	Ref. No.	Specifications
Ni Seplife IDA55 S	H4013102	25ml
	H4013103	100ml
	H4013104	500ml
	H4013105	1L
	H4013106	5L
	H4013107	10L
Ni Seplife NTA55 S	H4023102	25ml
	H4023103	100ml
	H4023104	500ml
	H4023105	1L
	H4023106	5L
	H4023107	10L

Product Name	Ref. No.	Specifications
Ni Seplife IDA55 M	H4013202	25ml
	H4013203	100ml
	H4013204	500ml
	H4013205	1L
	H4013206	5L
	H4013207	10L
Ni Seplife NTA55 M	H4023202	25ml
	H4023203	100ml
	H4023204	500ml
	H4023205	1L
	H4023206	5L
	H4023207	10L

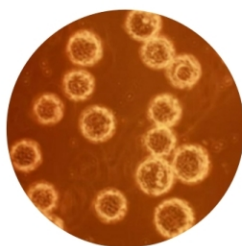


Seplife Microcarriers for Cell Culture

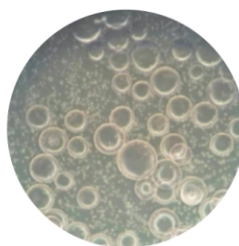
The cell culture microcarrier is a spherical carrier suitable for cell attachment growth with a dextran matrix. It is also widely used in industrialization and can adapt to the growth of a variety of cells.

Cell culture using microcarriers is currently recognized as the most promising large-scale animal cell culture technology, which has the advantages of both suspension culture and adherent culture, and is easy to scale up.

Sunresin's microcarriers are widely used in the large-scale production of biological products such as vaccines, monoclonal antibodies, viral vectors for gene therapy. The Seplife LX-MC-dex1 cell culture microcarrier is Sunresin's proprietary product suitable for almost all adherent cells. (such as Vero, CHO, 293T, MDCK, etc.) Cells can grow on the 2D surface of the microspheres. One gram of Seplife LX-MC-dex1 microcarrier provides 4400 cm² of surface area for cell attachment.



Cell culture



Cell digestion

Microcarriers have a wide range of applications. In addition to the coronavirus vaccines production, microcarriers are also used for vaccine productions for polio, rubella, rabies, influenza, Japanese encephalitis, respiratory syncytial virus (RSV) and foot-and-mouth disease (FMD), etc. Compared with other cell culture processes, microcarrier culture processes can increase yield, reduce costs and pollution. In addition, microcarriers can also be widely used in monoclonal antibodies, natural and recombinant proteins, and the promising field of immune cell therapy. I virus (RSV) and foot-and-mouth disease (FMD) vaccines, etc. Compared with other cell culture processes, microcarrier culture processes can increase yield, reduce costs, and reduce pollution. In addition, microcarriers can also be widely used in monoclonal antibodies, natural and recombinant proteins, and the promising field of immune cell therapy.

• Seplife LX-MC-Dex1 Technical Specifications

Particle Size (μm)		Swelling Factor (ml/g)	Microbial content (colony count/g dry weight)	Approximate number of microcarriers per gram (dry weight)
60-87μm (>70% , Dry powder)	145-240 (Saline)	17-22	< 100	4.3*10 ⁶

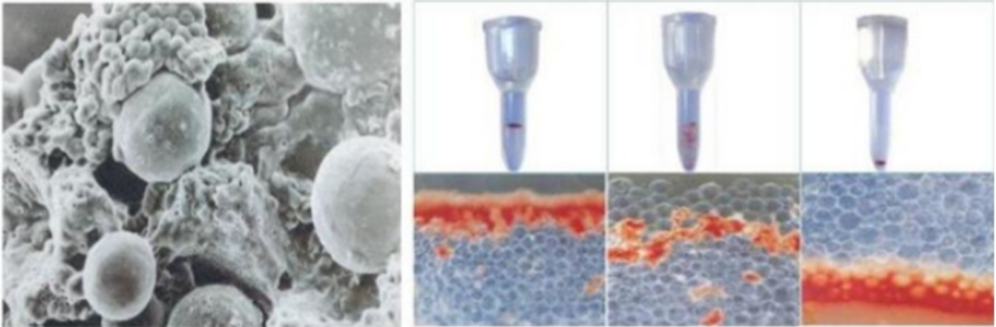
• Ordering information for Seplife microcarriers

Product	Ref. No.	Pack Size
Seplife LX-MC-Dex1	D6007310	25g
	D6007311	100g
	D6007312	500g
	D6007313	1kg
	D6007314	2.5kg
	D6007315	10kg

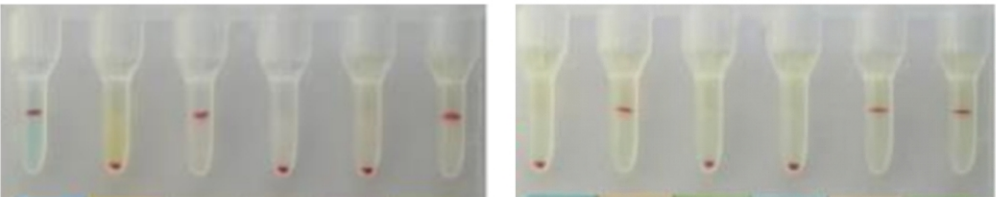
Annexe 1: Application of Dextran Gel Filtration Resins in Immunoassay

The micro-column gel immunoassay technology is simple, fast, accurate, sensitive, small in sample usage, long in storage time, and easily standardizable. Its main applications include: red blood cell antigen and antibody detection, platelet antigen and antibody detection, detection of multiple pathogenic microorganisms, detection of tumor markers, detection of neuroendocrine factors, etc.

Combining the interaction between antigen and antibody with size exclusion technology, the essence of the reaction is the Red Blood Cell agglutination test, which is also called microcolumn gel hemagglutination test. The micro-column tube is filled with dextran gel filtration resins. In the micro-column tube, the red blood cells and the corresponding antibodies are combined to form a Red Blood Cell agglutination. Under a certain centrifugal force, the agglutination is located on the surface or in the middle of the resins, but the red blood cells are not bound to the antibody and are still scattered red blood cells. Under the same centrifugal force, the red blood cells settle to the bottom of the microcolumn.



The Seplife Dextran Gel Filtration resins have high uniformity of particle size, low non-specific adsorption, and good stability. These features have been proved in microcolumn gel detection experiments.



Annexe 2: Precautions for the use of gel filtration chromatography

• Selection of columns

The selection of chromatography column size mainly depends on the amount of sample and the requirement of resolution. In general, the length of the chromatography column has a big impact on the resolution. Longer columns have a higher resolution than shorter ones; however, the length of the column should not be too long, otherwise it may cause difficulties such as column inhomogeneity and slow flow rate. Usually the column length should not exceed 100 cm, and to obtain high resolution, the columns can be used in series. Gel Filtration columns used for group separations, such as desalting columns, are generally shorter due to lower resolution requirements.

• Identification of columns

After the column is loaded, it should be uniform with no lines and no bubbles under observation with naked eye. Alternatively, a colored substance such as blue dextran-2000 or haemoglobin can be used on the column and the elution behavior of the colored zone in the column can be observed to test the homogeneity of the column. If the colored band is narrow, flat and falls uniformly, the resins in the column are well loaded and can be used; if the band is diffuse and distorted, the column needs to be reloaded. It is also worth mentioning that sometimes in order to prevent the adsorption of the sample on the new column, some substances can be used to pre-pass the column to avoid adsorption. At the same time, the efficiency of column should be tested.

• Selection of eluent

The principle of gel filtration chromatography is size exclusion. The mobile phase in gel filtration only acts as a carrier while the process is generally not dependent on changes in the nature and composition of the mobile phase to improve resolution, like in other chromatographic methods. The main purpose of changing the eluent is to eliminate interactions such as adsorption between the components and the stationary phase, so the choice of eluent for gel filtration is less stringent than for other chromatographic methods. Due to the simple separation mechanism of gel filtration and the wide pH range in which the media is stable, the selection of eluent depends mainly on the sample to be separated and in general, any buffer that can dissolve the eluted material without denaturing it can be used for gel filtration. To prevent possible adsorption to the media, the eluent generally contains a certain concentration of salt.

• Sampling volume

Samples should be loaded as fast and evenly as possible. The amount of the sample loaded may have a big impact on the chromatographic result. Too much sample loading will cause overlap of elution peaks and affect the separation effect; too little sample addition will result in a small amount and low concentration of each component after purification, resulting in low experimental efficiency. The amount of sample depends on the specific experimental requirements. In general, a big column corresponds to a big sample. If the difference of the molecular size in the sample is big, the sample volume can also be big. Generally speaking, the sample volume for fractional separation is about 1%-5% of the volume of the column bed, while the sample volume for group separation can be larger, generally about 10%-25% of the volume of the column bed.

If available, a small sample volume can be used for the first analysis and a suitable sample volume can be selected according to the elution peak. Suppose the elution volumes of the two components to be separated are V_{e1} and V_{e2} respectively, then in theory, the sample volume should not exceed $(V_{e1}-V_{e2})$. In reality, the sample volume should be less than this value due to sample diffusion. If the elution peaks of the components to be eluted are well separated, then the sample volume can be increased to improve efficiency; if they are just separated or not completely separated, the sample volume should not be increased or even reduced. In addition, it is important to note that the insoluble material in the sample must be removed before the sample is loaded to avoid contamination of the column. The viscosity of the sample should not be too high, otherwise it will affect the separation effect.

• Speed of elution

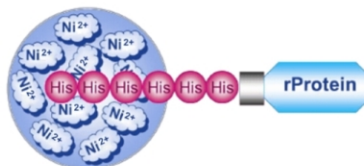
The speed of elution will also affect the separation effect of gel filtration. Generally, the elution speed should be constant and appropriate. There are usually two methods to keep the elution rate constant, one is to use a constant flow pump, and the other is to use constant pressure gravity elution. The elution speed depends on many factors, including column length, resin type, particle size, etc. Generally speaking, with a slow elution speed, samples can be fully balanced with the matrix, leading to a good separation effect. However, slow elution may increase the diffusion of the sample and widen the zone, which leads to a reduced resolution and a prolonged experiment time; therefore, the appropriate elution speed should be selected according to the actual situation in the experiment. Preliminary experiments can be carried out to decide the elution rate. Usually the producer of the Gel Filtration resins provides a recommended flow rate for reference.

In conclusion, the various conditions of gel filtration chromatography, including media type, column size, eluent, sample amount, elution speed, etc., should be selected according to specific experimental requirements. For example, if the difference between the components in the sample is small, the experiment requires gel filtration to have a higher resolution. The options for increasing the resolution should mainly include: choose a media that includes each component to be separated but the separation range is as small as possible; choose a media with small particles; choose a media with high resolution; choose longer, larger diameter columns, reduce the sample volume, reduce the elution speed, and so on. But as mentioned before, there is a limit to various options, and exceeding this limit may have an opposite effect. Another point that needs mentioning is that prior to the experiment, it is recommended to refer to relevant experiments and literature as much as possible and conduct pre-experiments to select the most suitable experimental conditions.

Annexe 3: Purification Guidelines for HIS-Tagged Proteins

• Principle of HIS- tagged protein purification

Histidine (HIS) has an imidazole group on its residues which selectively binds to metal ions by forming ligand bonds with transition metal ions such as Ni^{2+} and Co^{2+} . These metal ions can be immobilized on the chromatography resin with chelating ligands, so that proteins with a histidine tag can be selectively bound to the resin when passing through, whereas other impurity proteins cannot or can only be weakly bound. The HIS-tagged protein bound to the resin can be competitively eluted by increasing the concentration of imidazole in the buffer, resulting in a high purity HIS-tagged protein.



• Optimization strategy for the purification of HIS-tagged proteins

Optimization of the HIS-tagged protein purification process is based on changing the strength of the ligand binding force between the tag protein and the metal ion. This binding force is mainly related to the following factors.

- **Tag length and level of exposure** The most common polyhistidine tags (HIS- tags) are formed of six histidine residues. However, in practice, the number of histidines residues can vary from four to ten depending on the situation. Shorter tags bind weaker to metal ions. Longer tags bind stronger to metal ions. The impact of the level of exposure of the tag is easily understood.
- **Metal ion radius** In addition to the most commonly used Ni^{2+} , metal ions such as Cu^{2+} , Zn^{2+} and Co^{2+} can also be used in the purification of HIS- tag proteins, with different metal ions having different radius and different binding abilities. The smaller the ion radius, the stronger the binding capacity to the HIS- tag. The binding ability of the metal ions to the HIS- tag is as follows: $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+}$
- **Buffer conditions** HIS- tag proteins have stronger binding capacity with metal ions under neutral or weakly basic conditions than under acidic conditions. They have stronger binding capacity in phosphate buffer than in Tris-HCl buffer. Imidazole can compete with HIS- tag proteins to bind metal ions, so increased concentration of imidazole in the buffer will result in reduced binding capacity. Some reagents that chelate Nickel ions, such as EDTA or citric acid, have a great effect on protein binding to metal ions and should be avoided. The addition of 0.01-0.5% tween or triton to the buffer can reduce non-specific adsorption due to hydrophobic interactions.
- **Contact time** The binding capacity of HIS- tag proteins to metal ions is naturally enhanced by increasing the contact time within a certain range.

• Some frequently encountered problems in Purification of HIS- Tagged Proteins

1. The HIS- tagged protein doesn't bind (Difficulty in adsorption)

- Whether the HIS- tag is missing

In the process of expressing foreign proteins in *Escherichia coli*, sometimes the sequence is lost. If the tag is lost, the protein does not bind. This can be detected by Western-Blot.

- Whether the tag is exposed to the surface after expression

Add 1-2M urea into the sample and equilibration buffer to loosen the protein structure and allow adsorption without denaturing the protein. For proteins that are inherently denatured, use 6M guanidine hydrochloride to expose the tag, if the 8M urea does not allow adsorption. If there is a disulphide bond, it is best to add 1-2 mM DTT to solve the problem. Alternatively, try to increase the length of the HIS- tag through the upstream, increase the number of exposed tags or add the locations for adding tags.

- What metal ion was chosen

If Ni^{2+} or Co^{2+} was chosen, it can be replaced with the more potent Cu^{2+} or Zn^{2+} .

- Buffer conditions

Properly increase the pH of the buffer, reduce the concentration of imidazole in the buffer, adjust the salt concentration in the buffer or change the buffer system. All of these methods may be helpful.

- Try to extend the contact time

2. The bound proteins cannot be eluted (Difficulty in elution)

- Increase the concentration of imidazole in the eluent or lower the pH for elution.
- Add 0.2% detergent TritonX-100 to the eluent for elution to eliminate non-specific adsorption between the protein and the media.
- Reduce the loading amount or perform a linear gradient elution with imidazole to reduce the protein concentration to avoid protein precipitation.

3. Insufficient protein purity

- Change the metal ions

The main reason for the lack of purity is that some of the natural HIS-containing proteins in the host protein are bound to the media. Then the protein purity can be improved by replacing with Co^{2+} , which has a weaker binding capacity, so that the impurity proteins containing HIS- tags cannot be bound.

- Optimize the binding and elution conditions

Figure out the most suitable buffer system and sample loading and elution conditions to separate the tagged proteins from the impurity proteins as much as possible.

- Double tag purification

The StrepII- tag is also a small length tag with 8 amino acids. It is possible to add both the HIS- tag and the StrepII- tag to the recombinant protein. Protein purity can be improved using two-step affinity chromatography.

Annexe 4: Principle and application of separation and elution using Seplife LH-20 resins

As a special purification medium, Seplife LH-20 produces different separation modes when separating certain groups of compounds in different mobile phase systems.

• Seplife LH-20 in water-based separation system

The difference in polarity and the slight change in solubility lead to differences in the residence time of various water-soluble substances. The adsorption effect determines the important characteristics of the LH-20 water separation system for the selective separation of substances. The separated substances are usually eluted in order of molecular sizing

The water separation system using Seplife LH-20 is typically suitable for separating various inorganic salts. For organic salts, the difference in solubility is not the main basis for separation, and the elution order is mainly determined by the anions in the salt.

• Seplife LH-20 in methanol-based separation system

In addition to water, methanol has been regarded as the most common polar mobile phase for many years. Features of methanol separation system include the following:

- When in operation, the retention of aromatic and heterocyclic compounds is stronger than the retention of aliphatic and cycloaliphatic compounds.
- The methanol separation system using Seplife LH-20 can also differentially retain different kinds of substances in aromatic compounds.

• Seplife LH-20 in acetone-based separation system

When acetone, an aprotic solvent, is used as the mobile phase of the Seplife LH-20 separation system, the separation is mainly through the difference in the hydroxyl group and carbonyl group carried by the compounds. Therefore, it is very different from the highly polar methanol-based separation system

• Seplife LH-20 in dichloromethane -based separation system

When dichloromethane is used as the mobile phase, the separation of compounds is mainly based on two factors:

- Difference in solubility of compounds in dichloromethane ;
- The slight difference in polarity and alkalinity between substances.

• Seplife LH-20 in ethyl acetate-based separation system

Ethyl acetate, a medium-polar aprotic solvent, is better than acetone and dichloromethane in the separation of certain low-molecular-weight organics. In the elution sequence of the mixture, ethyl acetate is the same as acetone and dichloromethane, but the column bed volume of the stationary phase used is smaller and the separation speed is greater. In addition, the swelling degree of Seplife LH-20 in ethyl acetate is less than that in acetone. Therefore, the pore volume of the swollen gel becomes smaller and the pore diameter becomes narrower, making it easier to adsorb low-molecular-weight medium polarity organic compounds to achieve better separation effect. The principle of separation of mixtures with ethyl acetate is mainly based on the differences in polarity and hydrogen bonds between the substances.



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