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Agarosix-SP Excel Resin User Manual

1. Product Introduction

Agarosix-SP Excel is a strong cation exchange (SCX) chromatography resin, specifically designed for the purification of complex biomolecules. The resin is based on an agarose matrix with long-chain dextran, offering high biocompatibility and excellent physicochemical stability.

For samples requiring strict biocompatibility, the highly hydrophilic agarose-based matrix minimizes nonspecific adsorption, ensuring efficient purification. Agarosix-SP Excel is functionalized with sulfonic acid groups covalently bonded to the agarose beads, forming a high-capacity strong cation exchange medium.

Compared to Agarosix-SP FF, Agarosix-SP Excel offers higher pressure resistance and greater binding capacity, delivering enhanced separation efficiency and higher recovery rates for biomolecules.

Product Characteristics

High binding capacity and excellent biocompatibility

High resolution, high column efficiency, and high recovery rate

High batch-to-batch reproducibility and easy scalability

2. Safety

For information on the safe use of this product, please refer to the Safety Data Sheet (SDS).

3. Product Properties and Application

3.1. Chromatography Media Chemical Structure and Technical Parameters

Agarosix-SP Excel is a strong cation exchange chromatography medium, featuring sulfonic acid ($-SO_3^-$) groups as functional ligands. The structural schematic is shown in Figure 1, and the detailed product technical specifications

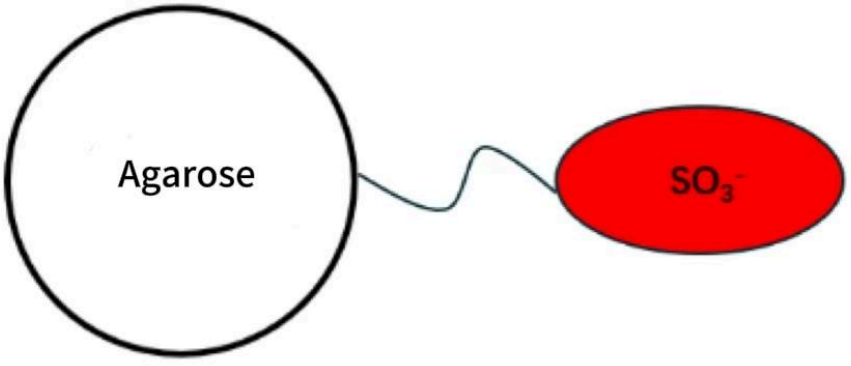

Figure 1. Schematic Diagram of Chromatography Media Ligand Structure

Table 1. Technical Parameters of Agarosix-SP Excel Chromatography Media

Product Name	Agarosix-SP Excel
Ion Exchange Type	Strong Cation Exchange (SCX)

Matrix	Agarose with long-chain dextran
Functional Group	Sulfonic Acid (-SO_3^-)
Average Particle Size	~90 μm
Ion Exchange Capacity (eq/L)	0.16 - 0.25 eq/L
Flow Rate/Pressure Relationship*	1000 cm/hr (operating pressure: 1.5 bar)
Dynamic Binding Capacity (/mL Resin)*	≥100 mg LYS per mL resin
pH Stability (Operation) pH Stability (Cip)	2 - 13 1 - 14
Operating Temperature	4-30 ℃
Operating Pressure	≤ 0.3 MPa (3.0 bar)
Chemical Stabilities	Compatible with common aqueous buffers Stable in: 1.0 M NaOH DMSO DMAC 6.0 M Guanidine Hydrochloride 30% Isopropanol 70% Ethanol
Storage Conditions	Refer to **Section 8: Product Storage**
Transportation Conditions	4-35 ℃, stored in 20% ethanol

*Note:
1. The dynamic binding capacity (DBC) was tested using 1.0 mg/mL lysozyme in 50 mM phosphate buffer (pH 6.0) with a 2-minute residence time and 10% breakthrough threshold.
2. Flow rate and pressure tests were conducted with a 200 mm column height at 2 bar operating pressure.
3. The solvent stability test confirmed that after one week at 40°C, the resin retained ≥90% of its original binding capacity.

4. Column installation for chromatography

The Agarosix MC90-MMC chromatography medium requires different column packing methods depending on the application scale. Laboratory-scale packing differs significantly from large-scale production packing due to variations in column dimensions, flow rates, and operational requirements.

4.1. Laboratory scale column packing (ID 6.6 mm - 25 mm)

4.1.1. Preparation

4.1.1.1. Packing equipment: Ensure the FPLC system functions normally, especially the pressure detection module and conductivity detection module.

4.1.1.2. Solution preparation: Prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.1.2. Replace storage solvent

Agarosix-SP Excel resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with purified water. Quick Replacement Method: Mix the resin thoroughly, take the required amount of slurry, and pour it into the column tube. Install the column head and flush with purified water for 5 CV. Then, transfer the resin into a wide-mouth bottle and add purified water until the resin volume ratio reaches 50-70%. Alternatively, the storage solvent can be replaced using a sedimentation method.

4.1.3. Calculation of slurry ratio

Quick Calculation Method: Mix the slurry thoroughly until the mixture is homogenous, take 20 mL, and add it into a glass column such as a Generik FPLC 10×400 mm glass column. Open the bottom stopper to allow water to drain out until the resin settles and the bed height is stable. Measure the bed height using a ruler and calculate the resin volume. For example, if the bed height is 14 cm, the resin volume is calculated as 14×0.7854=10.9956mL, resulting in a slurry ratio of 54.98%. Alternatively, the slurry

ratio can be calculated using an overnight sedimentation method in a graduated cylinder with a resin settling time of 14–16 hours.

4.1.4. Calculation of slurry volume

$V_{slurry} = V / P = S \times H \times F / P$

V_{slurry}: Required slurry volume

V: Target column volume

P: Slurry ratio

H: Target column height

S: Cross sectional area of column

F: Compression factor

For example, if the cross-sectional area of a column with an inner diameter of 10mm is 0.7854 cm^2 and the target bed height for column is 20 cm, the required volume of resin is: V_{slurry} = 0.7854 cm^2 x 20 cm x 1.15/54.98% = 32.9 mL

*Note: For laboratory-scale column packing using saline conditions, the resin amount is calculated based on a 1.15 compression factor

4.1.5. Packing steps

4.1.5.1. Use a pipette to add 32.9mL of slurry to a Generik FPLC 10 x 250mm AF chromatography column tube (using a column mounting ring).

4.1.5.2. Start the flow at 100 cm/h and tighten the top adaptor onto the column body. Compress the resin by increasing the linear flow rate stepwise to 100 cm/h, 200 cm/h, 300 cm/h, etc., maintaining each step for 3.0 minutes. Continue this process until the column pressure reaches 3.0 Bar, then hold for 15 minutes. Turn off the chromatography system, then lower the top adaptor to the target height using a 1:1.04 compression factor (based on the marked bed height). Column packing is complete.

4.2. Pilot scale column packing (ID 100 mm - 300 mm)

4.2.1. Preparation

4.2.1.1. Location: Clean and dust-free, temperature of 18℃ -35℃, humidity of 45% -65%.

4.2.1.2. Packing equipment and pipelines: Wash the FPLC system and pipelines thoroughly, connect all pipelines, and check whether there is any leakage in the pipelines. If necessary, perform leak tests and verify that all parameters including pressure are displayed correctly.

4.2.1.3. Air Removal: Clean the column thoroughly and remove any bubbles from the upper and lower frits for later use.

4.2.1.4. QC testing equipment: Low pressure chromatography system.

4.2.1.5. Solutions: prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.2.2. Replace storage solvent

Agarosix-SP Excel resin is supplied in 20% ethanol at a 50% volume ratio. Before column packing, the 20% ethanol must be replaced with purified water. For manually packed columns with an inner diameter of 100 mm to 300 mm, the replacement method is as follows: mix the resin, take the required slurry, pour it into the column tube, open the outlet valve to allow the liquid to drain out, and replace it three times with purified water

4.2.3. Calculation of slurry volume

$V_{slurry} = V/50\% = S \times H \times F/50\%$

V_{slurry}: Required Slurry Amount
V: Target column volume
H: Target bed height
S: Cross sectional area of column
F: Compression factor
For example, if the cross-sectional area of a column with an inner diameter of 200mm is 314cm^2 and the target height for column is 18 cm, the required volume of slurry is V_{slurry} = 314 cm^2 x 18 cm x 1.20/50% = 13.6 L
*Note: Compression factor of 1.20 is applied, resin is stored in 20% ethanol.

4.2.4. Specific Operating Procedure

4.2.4.1. As described in the replacement of storage solvent process, resuspend the slurry, then close the bottom valve and allow the resin to settle naturally. Once the gel surface has dropped more than 5.0 cm, install the column head with degassed solution, tighten the sealing ring, open the bottom valve, and start the low-pressure chromatography system. Using 0.1 M NaCl aqueous solution as the mobile phase, accelerate resin settling at a linear velocity of 100 cm/h. Once the column bed has stabilized, mark the bed height.

4.2.4.2. Turn off the chromatography system, wait for the column pressure to drop to zero, then close the bottom valve. Rotate the four-way valve on the column head to the drainage tube. Lower the column head to the target height using a 1:1.13 compression factor (based on the marked bed height). Column packing is complete.

4.3. Production scale column packing (ID 450mm and above, automatic column packing)

4.3.1. Preparation

4.3.1.1. Location: Clean and dust-free, temperature of 18℃-35℃, humidity of 45%-65%.

4.3.1.2. Packing equipment and pipelines: Wash the FPLC system and pipelines thoroughly, connect all pipelines, and check whether there is any leakage in the pipelines. If necessary, perform leak tests and verify that all parameters including pressure are displayed correctly.

4.3.1.3. Air Removal: Clean the column thoroughly and remove any bubbles from the upper and lower frits for later use.

4.3.1.4. QC testing equipment: Low pressure chromatography system.

4.3.1.5. Solutions: prepare 0.1 M NaCl aqueous solution and 1 M NaCl aqueous solution.

4.3.2. Replace storage solvent

Agarosix-SP Excel resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, 20% ethanol must be replaced with purified water. Calculate the required amount of resin using the method below, then transfer it into the slurry tank. After settling, decant the supernatant and add an equal volume of purified water. Mix thoroughly and allow it to settle again. Repeat the replacement process three times.

4.3.3. Calculation of slurry volume
Vslurry = V/50% = S×H×F/50%
V: Required slurry volume
V: Target column volume
H: Target column height
S: Cross sectional area of column
F: Compression factor
For example, if the cross-sectional area of a column with an inner diameter of 600 mm is 2826 cm2 and the target bed height for column is 18 cm, the required volume of slurry is

Vslurry=2826 cm2 x 18 cm x 1.20/50% ≈ 122 L.
*Note: The resin is stored in 20% ethanol solution, and the required amount of resin is calculated using a compression factor of 1.20.

4.3.4. Packing steps

Eliminate air bubbles in the resin pipeline.

Start the pump and set the linear velocity to 300 cm/h to draw the required amount of resin.

After resin transfer is complete, flush the resin remaining in the pipeline with water.

Column Compression: Lower the column head at a linear velocity of 60 cm/h to compress the resin. When the column head is 2–3 cm above the resin surface, record the resin bed height and compress the column bed using a compression factor of 1.12.

4.4. Reference standards for column efficiency testing and evaluation

After packing the Agarosix-SP Excel column, the column efficiency testing method and evaluation standards can be referred to in Table 2.

Sample	1.0 M NaCl
Sample Volume	1.0-2.0% CV
Mobile Phase	0.1-0.5 M NaCl
Flow Rate	60-180 cm/h
Detector	Cond.
Qualified Standards	Asymmetry: 0.8-1.8; Theoretical plate number: ≥ 2000/m

Table 2. Column Efficiency Testing Methods and Evaluation Standards

4.5. Solutions to non-ideal column efficiency

4.5.1. When a tailing peak occurs, solutions include:

Reducing slurry concentration: Decrease the overall volume ratio of the resin.

Increasing packing flow rate: Raise the maximum packing pressure.

4.5.2. When a fronting peak occurs, the solutions are the opposite of those for tailing peaks.

4.5.3. For low column efficiency: Repacking the chromatography column and reducing the testing flow rate.

4.5.4. For peak splitting: Cleaning or replacing the frit and checking the test sample.

4.5.5. For column cracking: Increasing packing pressure during column packing, ensuring the mobile phase is degassed, and fully removing bubbles when connecting the column head.

5. Overview of Purification Method Optimization

During the actual purification process, the following common issues may arise. Table 3 provides recommendations for common problems and their solutions. These suggestions can help identify and resolve issues, ensuring smooth method development or production.

Table 3. Common issues in purification and troubleshooting methods

common problem	resolvent
High back pressure during operation	Check for system blockage, troubleshoot section by section, or contact the manufacturer; clean or replace the column frit; perform CIP to remove contaminants from resin pores.
Low Binding Capacity	Ensure the sample pH is 7-9 and conductivity <6 mS/cm; check column packing quality and test column efficiency and symmetry.
Low recovery rate	Avoid overloading by setting the sample load to 80% of the dynamic binding capacity; calibrate instrument flow rate; optimize elution pH and conductivity.
High CIP peak	Optimize elution conditions and improve CIP efficiency.
Resin adhesion	Wash with 0.5 M NaCl or 0.5 M NaOH to resolve adhesion issues.

6. Cleaning In Place (CIP)

If impurities are not removed through the regeneration process, leading to column blockage, increased back pressure, or reduced flow rate, online cleaning in either the forward or reverse direction can be performed to restore column performance. Since online cleaning typically increases column back pressure, it is recommended to use a linear flow rate no more than 50% of normal operating conditions. The specific cleaning method should be determined based on the nature of the impurities:

General impurities:
Wash the column with 5 column volumes of 0.5 M NaOH, then proceed with storage or equilibration.

Precipitated or denatured protein impurities:
Wash the column with 5 column volumes of 1.0 M NaOH. If cleaning is insufficient, use 1.0 M NaOH + 1.0 M NaCl, then proceed with storage or equilibration.

Strongly hydrophobic-bound impurities:
Wash the column with 2 column volumes of a non-ionic detergent (e.g., 0.1-1% Tween or Triton X-100), then immediately rinse with at least 5 column volumes of sterile-filtered equilibration buffer (pH 6.0-8.0). Alternatively, clean with 3-5 column volumes of 70% ethanol or 30% isopropanol, followed by purification with water to replace the organic reagent before storage or equilibration.

7. Sterilization

Since 20% ethanol or 10 mM NaOH storage solutions do not have bactericidal or endotoxin removal effects, it is recommended thatAgarosix-SP Excel be treated with 0.5-1.0 M NaOH for 0.5 to 1.0 hours before and during use to reduce the risk of microbial contamination.

8. Storage Guidelines

The product is sold with 20% ethanol as the storage solution. Upon receiving the resin, please store it under the following conditions:

Unopened resin: Store at 4-35°C in a sealed packaging container, with a shelf life of 60 months.

Used resin:

1) Storage in the chromatography column: After use, flush the column with 3-5 column volumes (CV) of 20% ethanol or 10 mM NaOH, then store it in a sealed condition at 4-35°C. To prevent ethanol evaporation and microbial growth, it is recommended to replace the storage solution every two months. Since organic solvents, alkalis, and purified water may affect the column material, and prolonged storage in the column may cause the resin bed to dry and crack, long-term storage in the column is not recommended.
2) Storage after column disassembly: Before disassembly, the column should undergo routine regeneration and sterilization procedures. Flush with 3-5 CV of sterile injection-grade water, followed by 3-5 CV of 20% ethanol as the storage solution. Remove the resin and transfer it to a clean and sterilized storage container. Add 20% ethanol storage solution, ensuring the storage solution volume is approximately equal to the resin volume. Store sealed at 4-35°C.

9. Disposal and Recycling

Since theAgarosix-SP Excel resin is difficult to degrade naturally, it is recommended to dispose of it through incineration or by outsourcing to a third-party disposal service.

10. Ordering Product

Product Name	Type	Particle Size	Product Number
Agarosix-SP Excel	Strong Anion exchange	90 µm	253390990

Prepacked column specifications: 4.2mL, 5.0 mL; Packaging specifications: 1 L, 5 L, 10 L, 50 L

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