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Monomix HC60-DEAE Excel Resin User Manual

1. Product Introduction

Monomix HC60-DEAE Excel weak anion exchange chromatography media is designed for the separation and purification of biological samples. It uses hydrophilic polymethyl methacrylate as the matrix, with a particle size of 60 μm, ensuring good physical and chemical stability.

The surface of Monomix HC60-DEAE Excel chromatography media is specially treated to enhance its hydrophilicity, minimizing non-specific adsorption with biological samples. Through proprietary surface modification techniques, ion exchange functional groups (DEAE) are bonded to the hydrophilic matrix, resulting in a weak anion exchange chromatography media with a high density and uniformity of the ion exchange surface layer.

Monomix HC60-DEAE Excel ion exchange chromatography media is widely applicable for the separation and purification of biomolecules such as recombinant proteins, vaccines, antibodies, and other biological samples.

Product characteristics:

High binding capacity and excellent biocompatibility

Rigid matrix can withstand high pressure and high flow rates

High resolution, high column efficiency, and high recovery rate

High batch-to-batch reproducibility and easy scalability

Production capacity: > 100 L

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

Monomix HC60-DEAE Excel is a weak anion exchange chromatography media with a diethylamino group as the functional group. The structural schematic is shown in Figure 1, and the specific product technical parameters are provided in Table 1.

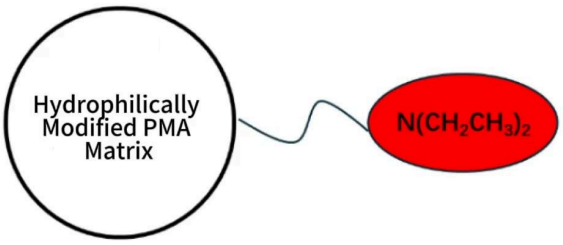

Figure 1. Schematic structure of the chromatography media functional group

Table 1. Technical Parameters of Monomix HC60-DEAE Excel Chromatography Media

Product Name	Monomix HC60-DEAE Excel
Ion Exchange Type	Weak Anion
Functional group	-N(CH_2CH_3)$_2$
Particle size (μ m)	~60
Ion Exchange Capacity (eq/L)	0.08–0.18
Dynamic Binding Capacity (/mL Resin)*	80–120 mg BSA
Flow Rate/Pressure Relationship*	900 cm/hr (Operating Pressure 2 bar)
PH stability (operation) PH stability (CIP)	2 - 13 1 - 14
Operating Temperature	4 - 35 ℃
Pressure Tolerance	≤ 1.0 MPa (100 bar)
Chemical stability*	Compatible with common aqueous buffers; other solvents include 1.0 M NaOH, DMSO, DMAC, 6.0 M guanidine hydrochloride, 30% isopropanol, 70% ethanol, etc.
Storage Conditions	Please refer to "8. Product Storage" for details
Transportation conditions	4-35 ℃, stored in 20% ethanol

Note:

1. DBC Testing Method: Linear flow rate of 360 cm/h, sample solution consisting of 2.0 mg/mL BSA in 50 mM phosphate buffer (pH = 8.0).
2. Pressure and Flow Rate Testing Method: Column height 200 mm, operating pressure 2 bar.
3. The resin was soaked in other reagents listed in the table at 40℃ for one week, and the results showed that the binding capacity remained above 90% of the original capacity.

3.2. Monomix HC60-DEAE Excel Purification Application

3.2.1. Purification Experiment for a Bovine Serum Albumin (BSA) Sample

Chromatography Column Information:

Monomix HC60-DEAE Excel (6.6 × 200 mm, CV = 6.842 mL)

Detector: UV 280 nm

Sample: Bovine serum albumin (BSA) sample

Purification steps	mobile phase	Residence time min	Rinse Volume (CV)
Alkali Wash	0.5 M NaOH	5	3

Column Wash	3% HAc	5	3
Equilibration	20 mM HAc-NaAc, pH5.7	5	5
Sample Load	Sample solution	5	—
Post-Equilibration	20 mM HAc-NaAc, pH5.7	5	5
Elution	22% 500 mM NaAc-HAc, pH 5.7	5	10
regenerate	100% 500 mM NaAc-HAc, pH 5.7	5	3
CIP	0.5 M NaOH	5	3
Equilibration	3% HAc	5	2
Storage	20mH HAc-NaAC, pH5.7	5	3

3.2.2. Purification Results for a Bovine Serum Albumin (BSA) Sample

Monomix HC60-DEAE Excel demonstrates high resolution. In the purification chromatogram (Figure 2), the target compound is observed in the elution step, while HCP, endotoxins, and contaminants are concentrated during the regeneration phase. This effectively increases the purity of the target product to 98.21%, with a recovery rate of 86.19%.

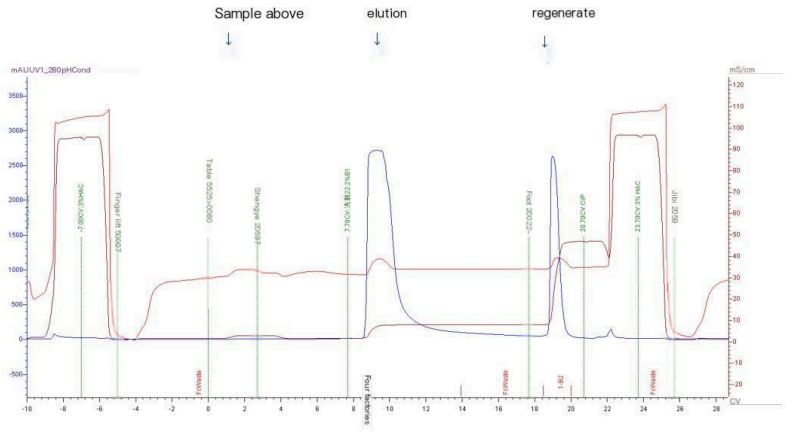

Figure 2. Purification Chromatogram of Bovine Serum Albumin (BSA) Sample Using Monomix HC60-DEAE Excel

4. Column installation for chromatography

Chromatography media require different packing methods for various scenarios. The packing method in the laboratory differs significantly from that used for large-scale production. Packing is related to factors such as pressure, flow rate, and other variables, and attention should also be paid to pressure changes during packing.

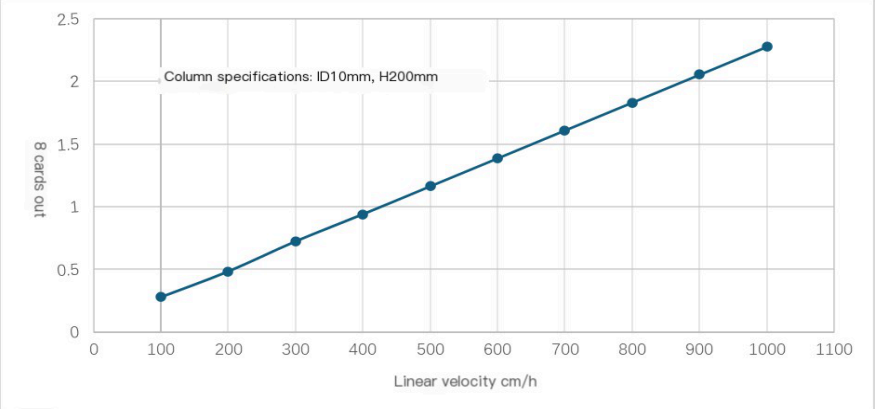

Figure 3. Monomix HC60-DEAE Excel Pressure-Flow Rate Curve

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

Monomix HC60-DEAE Excel resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, the 20% ethanol must be replaced with 0.1M NaCl. 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 0.1 M NaCl for 5 CV. Then, transfer the resin into a wide-mouth bottle and add 0.1 M NaCl until the resin volume ratio reaches 50-70%. Alternatively, the storage solvent can be replaced using a sedimentation method. Take an appropriate amount of slurry and place it into a centrifuge tube. Add approximately 5 mL of 1 M NaCl. Shake gently to mix and remove the floating foam. Let it sit for 10 minutes.

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

Monomix HC60-DEAE Excel resin is supplied in 20% ethanol at a 50% volume ratio. Before column packing, the 20% ethanol must be replaced with 0.1 M NaCl. 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 0.1 M NaCl.

4.2.3. Calculation of slurry volume

V_{slurry}= V/50% = S×H×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 1:13 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 1.0 M NaCl aqueous solution.

4.3.2. Replace storage solvent

Monomix HC60-DEAE Excel resin is stored in 20% ethanol at a 50% volume ratio when shipped. Before column packing, 20% ethanol must be replaced with 0.1 M NaCl. 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 0.1 M NaCl. 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 cm^2 and the target bed height for column is 18 cm, the required volume of slurry is

V_{slurry}=2826 cm^2 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

4.3.4.1. Eliminate air bubbles in the resin pipeline.

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

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

4.3.4.4. 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 Monomix HC60-DEAE 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.
Acceptance Criteria	Tail Peak Factor: 0.8-1.8 Column Efficiency: ≥ 2000 /m

Table 2.Monomix HC60-DEAE Excel 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	1. Chromatography system blockage leading to high pressure: Perform segmental troubleshooting for blockage or contact the manufacturer. 2. Column frit blockage: Clean or replace the frit. 3. Resin pore blockage due to contaminants: Perform CIP operation.
Low Binding Capacity	1. Inappropriate sample pH and conductivity: Recommended sample pH is 7–9, Cond < 6 ms/cm. 2. Poor column packing: Early flow-through occurrence, check column efficiency and symmetry.
Low Recovery Rate	1. Overloaded sample: Measure dynamic binding capacity first and set the sample load to approximately 80% of the dynamic capacity. 2. Inaccurate sample flow rate volume: Calibrate the instrument flow rate. 3. Low elution pH and conductivity: Optimize the elution conditions.
Cip Peak Height	1. Insufficient elution conditions: Optimize the elution conditions. 2. Insufficient CIP: Optimize the CIP procedure.
Resin Wall Adhesion Issue	1. Solution: Use 0.5 M NaCl or 0.5 M NaOH solution for washing to resolve the wall adhesion problem.

6. Cleaning In Place (CIP)

The purpose of CIP is to remove stubbornly bound impurities, precipitates, or denatured proteins from the column and system. The accumulation of these impurities can degrade column performance and contaminate the resin. In severe cases, it may lead to column blockage and increased back pressure. Online cleaning, either in the forward or reverse direction, can help restore column performance. A routine CIP procedure prevents impurities from accumulating on the column, thereby maintaining the column's binding capacity and separation efficiency.

The specific online cleaning method should be selected based on the nature of the impurities:

General Impurities and Routine Cleaning

After each experiment, clean with 0.1-0.5 M NaOH for 3-5 CV.

After NaOH cleaning, it is recommended to rinse with purified water, followed by equilibration buffer to quickly reduce pH.

Cleaning of Hydrophobic Impurities

Wash with 3-5 CV of 70% ethanol or 30% isopropanol.

If further cleaning is required, use 0.5 M NaOH + 30% isopropanol under strong cleaning conditions.

Use a low flow rate during cleaning (approximately half of the working flow rate).

When switching between high-salt buffers and organic solvents, rinse the chromatography column with purified water to prevent precipitation due to salt crystallization.

Cleaning of Other Impurities

For precipitates in the chromatography column, wash with a denaturant such as urea or guanidine hydrochloride, followed by routine CIP cleaning.

7. Sterilization

Since the 20% ethanol or 0.1M NaOH storage solution does not have bactericidal or endotoxin removal effects, it is recommended to treat the chromatography column with 0.1-0.5 M NaOH for 0.5 - 1 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 0.1 M 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 the Monomix HC60-DEAE 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	Order number
Monomix HC60-DEAE Excel	Strong Anion Exchange	60 µm	285160950

Pre-installed column specifications: 5.0 mL;
Packaging specifications for chromatography media: 1.0 L, 5.0 L, 10 L, 50 L.

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