Sepax Technologies, Inc.
5 Innovation Way, Newark, DE 19711, USA
Tel: (302)366-1101. Fax: (302)366-1151.
Toll-free: 1-877-SEPAX-US
www.sepax-tech.com

Sepax Monomix HC/MC Ion Exchange Resins

User Manual

Content

Sepax Technologies, Inc.
5 Innovation Way, Newark, DE 19711, USA
Tel: (302)366-1101. Fax: (302)366-1151.
Toll-free: 1-877-SEPAX-US
www.sepax-tech.com

I. Product Introduction

Sepax Monomix HC/MC chromatographic resins are specially designed for analysis and purification of biological molecules. The resins are composed of hydrophilic polymethacrylate base beads with high physical strength and chemical stability. The monosized resin has an average particle size of 15, 30 and 60 μm and an average pore size of 500 and 1000 Å. The resin surface is highly hydrophilic, which minimizes non-specific binding with biological samples. On the hydrophilic resin surface, strong cation exchange group (SP), strong anion exchange group (Q) and weak anion exchange group (DEAE) are covalently attached via proprietary technologies developed by Sepax. High mechanic stability ensures the resins tolerate high-pressure operation up to 10 bars, allowing for faster flow rate and shorter operation time. High chemical stability ensures the resins stand the acoustic cleaning procedure with a pH up to 14. These superior characters make the Sepax Monomix HC/MC IEX resins uniquely applicable at various bio-separation stages, from laboratory discovery, pilot-scale purification to industrial process.

Sepax Monomix HC/MC IEX resins are made with different linkers between the resin surface and the IEX functional groups. HC series are of much higher dynamic capacity than the MC series. The unique structural difference between these two types of resins offer different selectivity in the separation of biomolecules, such as peptides, insulins, proteins, antibodies, viruses, vaccines, heparins, and nucleotides.

Resin Structure

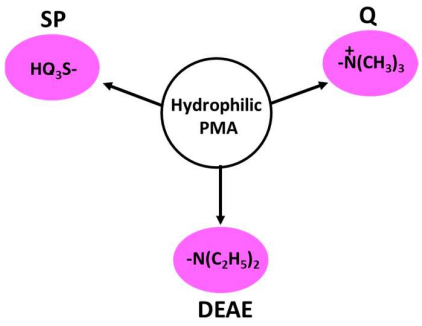

Figure 1. Monomix HC/MC IEX resin structures. Strong cation exchange functional group is sulfonic acid (SP). Strong anion exchange functional group is trimethyl quaternary amine (Q), and weak anion exchange group functional group is diethyl amine (DEAE).

Sepax Technologies, Inc.
5 Innovation Way, Newark, DE 19711, USA
Tel: (302)366-1101. Fax: (302)366-1151.
Toll-free: 1-877-SEPAX-US
www.sepax-tech.com

Resin Characteristics

- High binding capacity and excellent biocompatibility
- Capable of withstanding high pressure and high flow rate due to rigid base bead matrix
- High separation resolution, efficiency and recovery
- High lot-to-lot consistency
- Easy to scale up
- Highly hydrophilic surface with minimal non-specific binding
- Small volume change under normal packing conditions
- Supply capacity: >100L per order

Technical Specifications

Table 1. Monomix HC IEX Resins

Resin Type	Monomix HC-Q			Monomix HC-DEAE			Monomix HC-SP		
Matrix	Hydrophilic polymethacrylate								
Functional Group	$-N^+(CH_3)_3$			$-N(C_2H_5)_2$			$-SO_3H$		
Particle Size (μm)	15	30	60	15	30	60	15	30	60
Pore Size (Å)	1000			1000			1000		
DBC* (per mL resin)	≥ 95mg BSA	≥ 90mg BSA	≥ 80mg BSA	≥ 95mg BSA	≥ 90mg BSA	≥ 80mg BSA	≥ 110mg Lysozyme	≥ 90mg Lysozyme	≥ 90mg Lysozyme
Maximum Linear Flow rate (cm/h)	1000								
Operation Temperature (℃)	≤ 40								
pH range	2-12								
Maximum Pressure	≤ 1 MPa (10 bar)								
Compatible Mobile Phases	Compatible with aqueous solution, a mixture of water and acetonitrile, ethanol, etc. Typical buffers: Tris, phosphate, and acetate.								
Storage	70% (v/v) in 20% ethanol								
Regeneration	1-2 M NaCl								
CIP	0.5 M HCl or 0.5-1.0 M NaOH. Impurities with strong hydrophobic binding can be cleaned with 0.1-1% Tween or Triton X-100								

Table 2. Monomix MC IEX Resins

Resin Type	Monomix MC-Q			Monomix MC-DEAE			Monomix MC-SP		
Matrix	Hydrophilic polymethacrylate								
Functional Group	$-N^+(CH_3)_3$			$-N(C_2H_5)_2$			$-SO_3H$		
Particle Size (μm)	15	30	60	15	30	60	15	30	60
Pore Size (Å)	500			500			500		
DBC* (per mL resin)	≥ 50mg BSA	≥ 40mg BSA	≥ 30mg BSA	≥ 50mg BSA	≥ 40mg BSA	≥ 30mg BSA	≥ 60mg Lysozyme	≥ 40mg Lysozyme	≥ 30mg Lysozyme
Maximum Linear Flow rate (cm/h)	1000								
Operation Temperature (℃)	≤ 40								
pH range	2-12								
Maximum Pressure	≤ 1 MPa (10 bar)								
Compatible Mobile Phases	Compatible with aqueous solution, a mixture of water and acetonitrile, ethanol, etc. Typical buffers: Tris, phosphate, and acetate.								
Storage	70% (v/v) in 20% ethanol								
Regeneration	1-2 M NaCl								
CIP	0.5 M HCl or 0.5-1.0 M NaOH. Impurities with strong hydrophobic binding can be cleaned with 0.1-1% Tween or Triton X-100								

* Dynamic Binding Capacity (DBC) measurement conditions:

- HC/MC-Q: linear flow rate 180 cm/h, 2.0 mg/mL of BSA in 50 mM Tris buffer, pH 8.5
- HC/MC-DEAE: linear flow rate 180 cm/h, 2.0 mg/mL of BSA in 20 mM Tris buffer, pH 8.0
- HC/MC-SP: linear flow rate 360 cm/h, 1.0 mg/mL of lysozyme in 50 mM phosphate buffer, pH 6.0

II. Instructions for Use

2.1 Safety Precautions

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

2.2 Resin Preparation before Use

This product is normally shipped in an aqueous solution containing 20% ethanol and should be washed with deionized water before getting in contact with salt buffer

solutions. This can be accomplished by rinsing with 3 times column volume of deionized water and can be part of the column packing procedure (see Section 2.3.2 below).

2.3 Column Packing

2.3.1 Calculate column volume (CV): CV = column cross-sectional area (πr^2) x bed height (h), r is the column radius;

2.3.2 Gently stir the resin to completely disperse it to form a uniform slurry. Measure the required volume of the resin slurry and pour it into a clean transparent glass or plastic vessel. After natural sedimentation, decant 20% ethanol solution. Add 3 CV of deionized water, gently stir evenly and then settle for about 30 minutes, decant the supernatant, and repeat 3 times;

2.3.3 After removing the supernatant, add column packing buffer (0.5M NaCl solution) to make 60-70% resin slurry (volume based), stir evenly and soak the resin for more than 12 hours (overnight);

2.3.4 Measure about 1.1 times CV of the resin and gently stir evenly to make the final packing slurry. Pour it into a column with filter plate at the bottom to allow packing buffer to flow through and resin to settle steadily;

2.3.5 Place a flow distributor on the top of the column, press down the resin bed and connect to a pump;

2.3.6 Use 2-3 CV column packing buffer to flush the resin bed, compress resin beds at 2 times of normal working flow rate. The distributor position can be adjusted during the compressing process to ensure tightness of the resin bed. It is not recommended to use suction or gravity-only sedimentation to pack a column, especially for columns with a bed height of more than 10 cm;

2.3.7 Evaluation of column packing quality is carried out using a low molecular weight, unretained compound. The specific operating parameters are as follows:

Sample	1.0% (v/v) acetone aqueous solution	0.8 M NaCl
Sample Volume	1.0–2.0% of bed volume	1.0–2.0% of bed volume
Mobile Phase	Water or diluted buffer	Water or 0.5 M NaCl solution
Flow Rate	180 cm/h	180 cm/h
Detector	UV 280 nm	Conductivity
Specifications	Tailing factor: 0.8 – 1.5 30μm resin column efficiency: ≥4,000/m 60μm resin column efficiency: ≥2,000/m	

2.3.8 In case of non-ideal result, such as peak tailing, solutions include:
- Reduce the concentration of slurry

- Increase packing flow rate
- Extend packing time

If peak fronting occurs, solutions are the opposite of the above.

2.4 Column Use

2.4.1 Select and optimize a buffer system according to specific characteristics (isoelectric point, ion-exchange group, etc.) of targeted molecules to be separated/purified or analyzed;

2.4.2 Equilibrate the column with about 5 CV of buffer until conductivity and pH of effluent are constant, consistent with those of the fresh buffer;

2.4.3 **Sample preparation**: Solid samples can be dissolved in a buffer solution; low concentration samples can be dialyzed with a buffer to increase concentration; high concentration samples can be diluted with a buffer. Samples with insoluble impurities should be filtered first to avoid clogging the column and to prolong column life;

2.4.4 **Loading**: A sample loading volume should be determined according to resin loading capacity and purity of targeted molecule in crude sample; after loading is completed, continue to pump buffer until a stable baseline is obtained;

2.4.5 **Elution**: According to the characteristics of targeted molecule, choose a method (increasing the salt concentration or changing pH) to elute targeted molecule bound to the resin bed;

2.4.6 **Regeneration**: After each use of purification/separation, rinse the column with 1-2M NaCl solution to remove impurities adsorbed on the resin bed;

2.4.7 **Cleaning-in-Place (CIP)**: If impurities cannot be removed through the regeneration step, causing column clogging, increased back pressure, or decreased flow rate, the performance of the column can be restored by forward or reverse online cleaning – normally washed with 5 CV of 0.5 M NaOH. In general, online cleaning can result in increased back pressure of the column, so it is recommended to use linear flow rates within 0.5 times of normal operation conditions. Specific CIP methods are determined based on the characteristics of the impurities, some examples below:

a) For impurities such as precipitated or denatured substances, wash with 5 CV of 0.5-1.0 M NaOH; if the result is not satisfactory, use 0.5-1.0 M NaOH plus 1M NaCl, and then use at least 5 CV of 0.22um filtered buffer (pH6-8) to wash the column;

b) For impurities bound with strong hydrophobicity, wash the column with 2 CV of non-ionic detergent (such as Tween or Triton X-100 at a concentration of 0.1-1%), and then immediately use at least 5 CV of filtered buffer (pH6-8) to wash the column; it can also be washed with 3-4

CV of 70% ethanol or 30% isopropanol, and then at least 5 CV of filtered buffer (pH6-8) to wash.

III. Storage

Chromatographic resins that will not be immediately used should be stored in an aqueous solution containing 20% ethanol at 4 to 35 °C in a sealed container; resins that have packed in a column can be stored in an aqueous solution containing 20% ethanol at 4 to 35 °C.

IV. Ordering Information

Resin	Particle Size, Pore Size	Product Number	Package Size （L）	Prepacked Column (mL)
Monomix HC15-Q	15μm, 1000Å	280815950	0.5, 1, 5, 10, 100	1, 5
Monomix HC15-DEAE	15μm, 1000Å	280915950	0.5, 1, 5, 10, 100	1, 5
Monomix HC15-SP	15μm, 1000Å	280615950	0.5, 1, 5, 10, 100	1, 5
Monomix MC15-Q	15μm, 500Å	280415500	0.5, 1, 5, 10, 100	1, 5
Monomix MC15-DEAE	15μm, 500Å	280515500	0.5, 1, 5, 10, 100	1, 5
Monomix MC15-SP	15μm, 500Å	280215500	0.5, 1, 5, 10, 100	1, 5
Monomix HC30-Q	30μm, 1000Å	280830950	0.5, 1, 5, 10, 100	1, 5
Monomix HC30-DEAE	30μm, 1000Å	280930950	0.5, 1, 5, 10, 100	1, 5
Monomix HC30-SP	30μm, 1000Å	280630950	0.5, 1, 5, 10, 100	1, 5
Monomix MC30-Q	30μm, 500Å	280430500	0.5, 1, 5, 10, 100	1, 5
Monomix MC30-DEAE	30μm, 500Å	280530500	0.5, 1, 5, 10, 100	1, 5
Monomix MC30-SP	30μm, 500Å	280230500	0.5, 1, 5, 10, 100	1, 5
Monomix HC60-Q	60μm, 1000Å	280860950	0.5, 1, 5, 10, 100	1, 5
Monomix HC60-DEAE	60μm, 1000Å	280960950	0.5, 1, 5, 10, 100	1, 5
Monomix HC60-SP	60μm, 1000Å	280660950	0.5, 1, 5, 10, 100	1, 5
Monomix MC60-Q	60μm, 500Å	280460500	0.5, 1, 5, 10, 100	1, 5
Monomix MC60-DEAE	60μm, 500Å	280560500	0.5, 1, 5, 10, 100	1, 5
Monomix MC60-SP	60μm, 500Å	280260500	0.5, 1, 5, 10, 100	1, 5

For resin products or prepacked columns not listed above, please contact us.