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 Boronate Affinity Chromatography Resins

User Manual

Content

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I. Product Introduction

Sepax Monomix MC-Boronate affinity resins are designed to purify and analyze *cis*-diol containing compounds, such as glycoproteins, nucleic acids and sugars. The base beads are monosized, composed of hydrophilic polymethacrylate (PMA) of high physical and chemical stability, with *m*-Aminophenyl boronate covalently linked using a proprietary hydrophilic linker, as shown in **Figure 1**. This highly hydrophilic resin surface allows for minimized non-specific bindings with biological samples. The resin is currently offered with particle sizes of 30 and 60 µm and pore size of 1000 Å. The exclusion limit of dextran is approximately 2 x 10^6, and the exclusion limit of globular proteins is approximately 1 x 10^7, suitable for industrial purification. In addition, Sepax can provide high-resolution analytical chromatography columns under the product brand of Monomix MC-Boronate with a particle size of 10 µm and a pore size of 500 Å and 1000 Å.

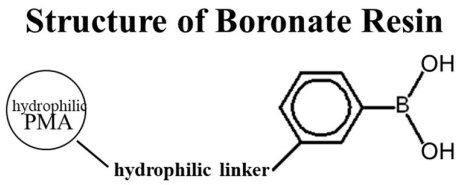

Figure 1. Chemical structure of Sepax Boronate affinity resin.

Purification Mechanism

Boronate binds to molecules with a *cis*-diol function group in a pH dependent way. The mechanism has been suggested that under mild basic conditions, coplanar *cis*-diol containing molecules bind to boronate to reversibly form a thermodynamically favored five-member ring, **Figure 2**. The binding is more stable at pH>7.5, while dissociation occurs at pH<6.5. The binding can be enhanced in the presence of Mg^{2+}. The diol containing molecules in the boronate complex can also be removed or eluted with other *cis*-diol reagents, such as sorbitol and mannitol.

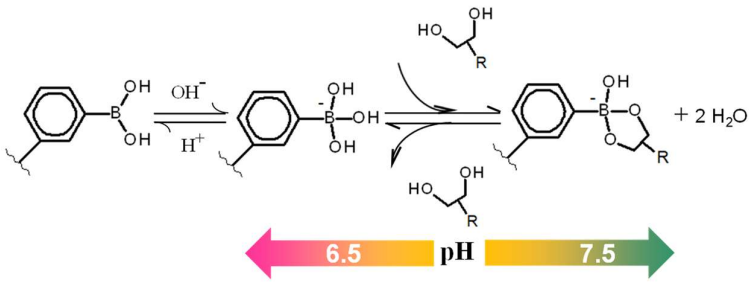

Figure 2. Mechanism of boronate binding to *cis*-diol containing molecules.

Resin Characteristics

- High binding capacity and excellent biocompatibility
- Capable of withstanding high pressure and high flow rate due to rigid base bead material
- 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

Resin	Monomix MC30-Boronate	Monomix MC60-Boronate
Base Bead Material	Hydrophilic polymethacrylate	
Particle Size (μm)	30	60
Pore Size (Å)	1000	
Boronate Loading (μmol/mL resin)	≥100	
Max Linear Flow Rate (cm/h)	1800	
Operation Temperature (°C)	⩽40	
pH Stability Range	2-12	
Operation Pressure	⩽1 MPa (10 bar)	
Mobile Phase Compatibility	1. Compatible with aqueous solution, water mixed with acetonitrile, acetone or methanol. Typical buffers: phosphate, acetate and HEPES, MES, primary amine-free salt buffer system. 2. The use of buffers pH<6.5 or *cis*-diol-containing reagents may affect adsorption.	
Long-term Storage	Store in 20% ethanol aqueous solution, 70% (v/v)	
Regeneration	Use 2.0 – 3.0 M NaCl	
CIP	Use 3-10 column volume of 0.1-0.5 M NaOH	

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 1.05 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

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. Normally it is a 0.01 M sodium phosphate buffer containing 0.1-0.2 M NaCl, and pH> 7.5. Generally, adsorption strength is inversely proportional to pH and ionic strength. A slight increase in ionic strength can help remove tightly bound contaminants. Non-ionic detergents (Tween®20, Triton®X, etc.) can be added to improve solubility;

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 to elute targeted molecule bound to the resin bed;

2.4.6 **Regeneration**: After each use of purification/separation, rinse the column with 2.0-3.0 M NaCl solution to remove impurities adsorbed on the resin bed; If other *cis*-diol-containing reagents are used, wash the column with 2 CV of 0.1 M acetic acid and equilibrate the column with loading buffer;

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.

III. Storage

Chromatographic resin that will not be immediately used should be stored in an aqueous solution containing 20% ethanol at 2 to 8 °C in a sealed container; resins to be used within 14 days can be stored in an aqueous solution containing 1 M NaCl at 2 to 8 °C in a sealed container.

IV. Ordering Information

Sepax Monomix MC-Boronate Affinity Resins

Product Name	Particle Size (µm)	Product Number	Package Size (L)	Prepacked Column (mL)
Monomix MC30-Boronate	30	283430950	0.5, 1, 5, 10, 100	1, 5
Monomix MC60-Boronate	60	283460950	0.5, 1, 5, 10, 100	1, 5

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