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Sepax Monomix MC SEC Chromatography Resins

Instructions for Use

Content

1. Safety Precautions

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

2. 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 3.2 below).

3. Column Packing

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

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;

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);

3.4 Measure about 1.2 times CV of the resin and gently stir evenly to make a final packing slurry. Pour it into a column with filter plate at the bottom (suggest using finer than 20 μm filter plate for 30 μm resins) to allow packing buffer to flow through and resin to settle steadily;

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

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;

3.7 Evaluation on 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	60 cm/h	60 cm/h
Detector	UV 280 nm	Conductivity

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.

4. Column Use

4.1 Select and optimize a buffer system according to specific characteristics (solubility, isoelectric point, etc.) of targeted molecule to be separated/purified or analyzed;

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;

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;

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;

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

4.6 **Regeneration**: After each use of purification/separation, rinse the column with 0.5-2M NaCl solution to remove impurities adsorbed on the resin bed;

4.7 **Cleaning-in-Place (CIP)**: If impurities are not removed by the regeneration step, they may cause blockage of the column, increased back pressure or decreased flow rate. The column performance can be restored by forward or

reverse online rinsing. A specific method can be established based on the characteristics of impurities:

a) For precipitated or denatured impurities, wash with 5 CV of 1 M NaOH (try 1 M NaOH + 1 M NaCl if results are not acceptable), followed by rinsing with at least 5 CV of 0.22 μm filtrated buffer (pH 6-8);

b) For impurities with strong hydrophobicity, wash the column with 2 CV of a non-ionic detergent (for example, Tween or Triton X-100 at a concentration of 0.1-1%), and then immediately rinse the column with at least 5 CV of filtrated buffer (pH 6-8); one can also wash with 3-4 CV of 70% ethanol or 30% isopropanol, and then immediately rinse with at least 5 CV of filtrated buffer (pH 6-8).

5. Storage

Chromatographic resin 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; the resin packed into a column can be stored in an aqueous solution containing 20% ethanol at 4 to 35 °C for a short period of time.