Column Care and Use Instructions

YMC-BioPro Ion Exchange Column

for Separation of Proteins, Nucleotides, and other Biomolecules

1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column for ion exchange chromatography. YMC-BioPro Ion Exchange Column is based on a newly developed hydrophilic polymer bead with a strong-anion exchanger (quaternary ammonium group) or a strong-cation exchanger (sulfopropyl group). A porous-polymer type [YMC-BioPro QA/SP] for high-performance and high-binding capacity and a non-porous-polymer type [YMC-BioPro QA-F/SP-F] for high-throughput and high-resolution analysis are available.

YMC-BioPro Ion Exchange Columns, which are manufactured under highly controlled conditions, must pass a series of stringent tests before being accepted for shipment (Please refer to the column inspection report). To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

2. Column Specification

Item	YMC-BioPro QA / YMC-BioPro SP	YMC-BioPro QA-F / YMC-BioPro SP-F					
Matrix	porous hydrophilic polymer beads	non-porous hydrophilic polymer beads					
Functional group	$-CH_2N^+(CH_3)_3$ / $-CH_2CH_2CH_2SO_3^-$	$-CH_2N^+(CH_3)_3$ / $-CH_2CH_2CH_2SO_3^-$					
Counter ion	Cl^- / Na^+	Cl^- / Na^+					
Particle size (μm)	5	5			3		
Column size length X I.D. (mm)	50 X 4.6	30 X 4.6	50 X 4.6	100 X 4.6	30 X 4.6	50 X 4.6	100 X 4.6
Flow rate (mL/min)	0.5 – 0.7	1.0 – 1.5	1.0 – 1.2	0.2 – 0.8	1.0 – 1.3	0.5 – 1.0	0.4 – 0.6
Max. flow rate (mL/min)	0.8	1.8	1.5	1.0	1.3	1.0	0.6
Max. pressure (MPa)	3.0	6.0	10.0	12.0	25.0	25.0	25.0
pH range	2 – 12	2 – 12			2 – 12		
Temp. range (°C)	4 – 60	4 – 60			4 – 60		
Column material	PEEK	PEEK			PEEK		

3. Recommendations for column connections, detector settings, and data processing considerations

- The "WP" at the end of the product code indicates the style of column endfittings. WP = Waters style
- Tubing must have flat ends and must bottom out in the column endfitting. Tubing must be connected to the column correctly to avoid creating a void between the column frit and tubing, which can cause a leak and result in poor column performance (e.g. peak tailing, loss of theoretical plate number).
- The shortest possible length of tubing with narrow inner diameters (tubing less than 0.15 mm, 0.006 inch I.D. is recommended) should be used for the connection from the injector to the column and from the column to the detector. Make sure not to have a gap in the connection.
- A sampling rate and a detector response (time constant) should be optimized. When using YMC-BioPro QA-F / SP-F for ultra-fast separation, we recommend a sampling rate of about 10 points per second or higher and a detector response of 0.5 s or faster to detect the sharp peak properly.

4. Mobile phase and sample solvent

- The shipping solvent is the following. It is as the same as the mobile phase as on the "COLUMN INSPECTION REPORT". When columns are not used for a long time, keep them in a cool place after replacing the below shipping solvent.

  Shipping solvent

  YMC-BioPro QA / QA-F: 20 mM Tris-HCl buffer (pH 8.1)

  YMC-BioPro SP / SP-F: 20 mM sodium phosphate buffer (pH 6.8)
- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- Recommendations of conditions for column use are shown in the specifications table in section 2. Avoid using a column repeatedly near the pressure limit or abrupt change in pressure to prevent shortening of the column life.
- Generally samples are adsorbed onto the top of the column with 20 – 50 mM of buffer as the first mobile phase, then eluted with a salt-concentration gradient method (sodium chloride concentration commonly adjusted between 0 to 0.5 M) or pH-gradient method. We recommend flushing the column with buffer containing about 1 M of sodium chloride for each run to remove residual impurities from the column with the final mobile phase.
- Water-soluble organic-solvent (maximum of 30%) Before adding such solvent, make sure that salt in the buffer will not precipitate. Other additives such as urea (≤8 M) or guanidine hydrochloride (≤6 M), which are commonly used as protein denaturants, nonionic surface-active agents, cationic surface-active agents (limited to YMC-BioPro QA/QA-F), or anionic surface-active agents (limited to YMC-BioPro SP/SP-F) are usable.
- Avoid solvents containing oxidant for the mobile phase.
- When possible, the sample should be dissolved in a solvent that is of the same composition as the initial mobile phase. Using a different buffer salts/additives concentration or pH solvent from the initial mobile phase for sample dissolution might result in the degraded in the binding capacity and distorted peak symmetry.
- To prevent exposure of the column to excessive pressures, the mobile phase and sample should be filtered through a 0.2 – 0.5 μm membrane filter. We recommend using a pre-column filter.

5. Column cleaning

A change of retention time or peak shape and/or pressure increase might result from the adsorption of fat-soluble substances or precipitated impurities in a sample. In such cases, follow these steps for column cleaning and regeneration. If these procedures will not solve the problem, then we recommend that you use new column

First replace the mobile phase to shipping solvent. Then inject 4 – 5 mL of following solvents ((1) – (4)) with running the initial solvents. We recommend using large size sample-loop (≥2 mL).

Cleaning solution

(1) 0.2 N NaOH aq／Acetonitrile（80／20）

(2) 1 M Acetic acid aq

(3) Nonionic surfactant (like 0.02% Brij™35) in initial mobile phase

(4) 6 M guanidine hydrochloride in initial mobile phase

Make sure the retention time or the peak shape is recovered after cleaning.

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