Column Care and Use Instructions

BioPro IEX Column, Accura BioPro IEX Column

for Separation of Proteins, Nucleotides, and Other Biomolecules

1. Introduction

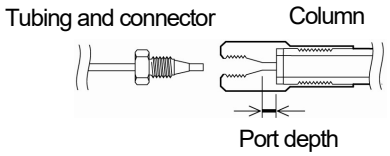
Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column for ion exchange chromatography. BioPro IEX Column and Accura BioPro IEX Column are based on a hydrophilic polymer bead with a strong-anion exchanger (quaternary ammonium group) or a strong-cation exchanger (sulfopropyl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

BioPro IEX Columns and Accura BioPro IEX 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. Recommendations for column connections, detector settings, and data processing considerations

- "WP" or "PTC" at the end of the product code indicates the style of column endfittings (see below for details).

Consideration of connector and endfittings



Item	The end of the product code	Column hardware specifications	Port depth	Style of endfittings
BioPro IEX	WP	PEEK	ca. 3 mm / 0.13 inch	Waters style (※)
Accura BioPro IEX	PTC	Bioinert coated stainless steel	ca. 2 mm / 0.09 inch	Parker style

※Use of a metal connector is not recommended. Inside parts of the column might be damaged if the metal connector is used.

- 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 BioPro IEX QF/SF and Accura BioPro IEX QF/SF 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.
- The correct direction of the solvent flow is indicated by an arrow on the column identification label.

YMC CO., LTD.
YMC Karasuma-Gojo Bldg., 284 Daigo-cho, Karasuma Nishiiru Gojo-dori, Shimogyo-ku, Kyoto 600-8106, Japan
TEL +81-75-342-4515 FAX +81-75-342-4550
URL:www.ymc.co.jp E-Mail:sales@ymc.co.jp

3. Recommendations for column use

・ Recommendations of conditions for column use are shown in the specifications table below. Avoid using a column repeatedly near the pressure limit, or with abrupt change in pressure to prevent shortening of the column life.

Item	品種	Particle size	Column size		Recommended flow rate (mL/min)	Flow rate limit (mL/min)	Pressure limit (MPa)	Usable temperature range
			I.D. (mm)	Length (mm)				
BioPro IEX	QA / SP	5 µm	4.6	30	0.5–0.8	1.0	2.5	4–60℃
				50	0.5–0.7	0.8	3.0	
				100	0.4–0.5	0.6	3.5	
	QF / SF	5 µm	4.6	30	0.5–1.0	1.5	6.0	
				50			10.0	
				100	0.5–0.8	1.0	12.0	
		3 µm	4.6	30	0.5–1.0	1.0	25.0	
				50				
				100	0.5–0.6	0.6		
Accura BioPro IEX	QF / SF	5 µm	4.6	50	0.5–1.0	1.5	10.0	4–80℃
				100		1.0	12.0	
				150			18.0	
				250			30.0	
			2.1	50	0.1–0.2	0.3	10.0	
				100		0.2	12.0	
				150			18.0	
		3 µm	4.6	50	0.5–1.0	1.5	25.0	
				100		1.0	25.0	
				150			30.0	
			2.1	50	0.1–0.2	0.2	15.0	
				100	0.1	0.1	15.0	
				150			20.0	

4. Mobile phase and sample solvent

・ Shipping solvents are listed in the table below. They are the same ones as the mobile phases indicated in the “COLUMN INSPECTION REPORT”. When columns are not used for a long time, keep them in a cool place after replacing with the shipping solvent.

Shipping solvent

BioPro IEX QA／QF Accura BioPro IEX QF	20 mM Tris-HCl buffer (pH 8.1)
BioPro IEX SP／SF Accura BioPro IEX SF	20 mM sodium phosphate buffer (pH 6.8)

・ 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.

・ Adjust the pH of the mobile phase in the range of 2 - 12.

・ 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 BioPro IEX QA/QF and Accura BioPro IEX QF), or anionic surface-active agents (limited to BioPro IEX SP/SF and Accura BioPro IEX SF) are usable.

- Avoid solvents containing oxidant for the mobile phase.
- When possible, the sample should be dissolved in a solvent that is the same composition as the initial mobile phase. Using a different buffer salts/additives concentration or a different pH solvent from the initial mobile phase for sample dissolution might result in decreased binding capacity and/or distorted peak shape.
- 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 a new column.

【Cleaning procedures】

1. Replace mobile phase with the shipping solvent.
2. Then inject 4 – 5 mL of following cleaning solutions A) – D) while running the shipping solvent. The cleaning is recommended to be conducted step-by-step and started from A). Column performance should be confirmed after cleaning with each solution. It does not need to proceed to another cleaning solution if one solution can restore the column performance. We recommend using a large-size sample-loop (≥2 mL).

Cleaning solution

A) 0.2 M NaOH aq／Acetonitrile（80／20）
B) 1 M Acetic acid aq
C) Nonionic surfactant (like 0.02% Brij™35) in the shipping solvent
D) 6 M guanidine hydrochloride in the shipping solvent

YMC CO., LTD.
YMC Karasuma-Gojo Bldg., 284 Daigo-cho, Karasuma Nishiiru Gojo-dori, Shimogyo-ku, Kyoto 600-8106, Japan
TEL +81-75-342-4515 FAX +81-75-342-4550
URL:www.ymc.co.jp E-Mail:sales@ymc.co.jp