Column Care and Use Instructions Accura BioPro IEX Column, BioPro IEX Column

Thank you for purchasing this product. To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

1. Specifications

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	Accura BioPro IEX QF BioPro IEX QF	Accura BioPro IEX SF BioPro IEX SF	BioPro IEX QA	BioPro IEX SP						
Matrix	hydrophilic non-բ	oorous polymer beads	hydrophilic porous polymer beads							
Charged group	-CH₂N⁺(CH₃)₃	-CH ₂ CH ₂ CH ₂ SO ₃ -	-CH ₂ N⁺(CH ₃) ₃	-CH ₂ CH ₂ CH ₂ SO ₃ -						
Counter ion	Cl-	Na⁺	Cl ⁻	Na⁺						
Usable temperature		cura BioPro IEX) (BioPro IEX)	4-60°C							
Column material		s steel (Accura BioPro IEX) BioPro IEX)	PEEK							

2. Shipping 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.

Column	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)		

3. Precaution for use

- · The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- 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).
- For BioPro IEX column, use of a metal connector is not recommended. Inside parts of the column might be damaged if the metal connector is used.
- 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 Accura BioPro IEX QF/SF and 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.
- 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.

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Column		Particle size	Colu I.D. (mm)	ımn size Length (mm)	Recommended flow rate (mL/min)	Flow rate limit (mL/min)	Pressure limit (MPa)
BioPro IEX	QA/SP	5 μm	4.6	30	0.5-0.8	1.0	2.5
				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
				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	30	0.1-0.4	0.5	15.0
				50	0.1-0.2	0.2	15.0
				100	0.1	0.1	15.0
				150			20.0

- 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 0–0.5 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 (Max. 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.

4. 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

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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
- \bullet Please note that specifications and appearance may change without notice.

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