Column Care and Use Instructions
BioPro HIC HT, BioPro HIC BF

for Hydrophobic Interaction Chromatography

1. Introduction

Thank you for purchasing a YMC high-performance liquid chromatography (HPLC) column for hydrophobic interaction chromatography. It is designed for separation of proteins and biopharmaceuticals such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs). BioPro HIC columns, which use non-porous polymer beads modified with butyl group, allow for fast and highly efficient separation.

BioPro HIC 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 the instruction carefully before using.

2. Specifications

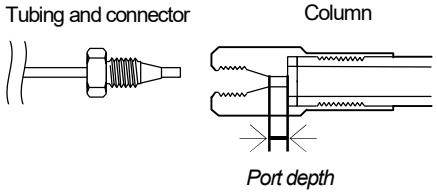
Item	BioPro HIC HT	BioPro HIC BF
Matrix	Hydrophilic non-porous polymer beads	
Bonded phase	Butyl	
Particle size	2.3 µm	4 µm
Recommended flow rate*	4.6 mmI.D: 0.5-1.5 mL/min (Maximum flow rate: 2.0 mL/min)	4.6 mmI.D.: 0.5-1.0 mL/min (Maximum flow rate: 1.2 mL/min)
Pressure limit	40 MPa	20 MPa
Usable pH range	2-12	
Usable temperature range	10-60 °C	
Column hardware	Stainless steel	

*Adjust flow rates based on the recommended flow rate so as to achieve better results under your conditions. Especially for columns with other diameters, the flow rates must be increased/decreased by the ratio of the cross-sectional areas. Note that continuous use near the pressure limit may result in short column life.

3. Column Installation

- The “PTH” or “WT” at the end of the product code indicates the style of column endfittings.

Consideration of connector and endfittings



The end of the product code	Port depth	Style of endfittings
PTH	ca. 2 mm / 0.09 inch	Parker style
WT	ca. 3 mm / 0.13 inch	Waters style

- In a proper tubing-column connection, the tubing has flat end and bottoms out in the column endfitting. If there is a void between them, leak may occurs and column performance may be negatively affected (e.g. peak tailing, loss of theoretical plate number).
- Use narrow internal diameter tubings leading to and from the column (tubing less than 0.15 mm, 0.006 inch I.D. is recommended) so that band spreading can be minimized.
- Optimize the sampling rate and the response time, if needed.
- Do not disconnect the column from the LC system before the pressure drops to zero.

4. Column Use

- BioPro HIC columns are shipped in 20% ethanol. The column can be stored in water or mobile phase for short term (overnight). For long term, store the column in 20% ethanol or methanol in water.
- The correct flow direction through the column is indicated by an arrow on the column identification label.
- Recommended operating conditions are shown in the table in section 2. Sudden pressure surge may cause the degradation of column performance. When a higher salt concentration buffer is used, make sure not to exceed the pressure limit.
- The columns are compatible with water-soluble organic solvents up to 30%. Make sure to confirm that there is no salt precipitation before using a mobile phase with organic solvent.
- To ensure the proper binding of the target materials to the resin, dissolve the sample in the starting mobile phase. If the sample precipitates at this salt concentration, dissolve the sample in the doubling dilution of the starting mobile phase with appropriate buffer or water.
- Filter the mobile phase and sample solution through a 0.2-0.5 µm filter to maintain the column performance. We also recommend a pre-column filter (XRPRCS03).

5. Column Cleaning

Most bound samples are eluted by washing the resin with salt-free buffer. However, when deterioration of the column such as increasing pressure is observed, more effective column cleaning procedure below is required.

1. Wash the column with water for 30 column volumes.
2. Inject 100-250 µL of 0.1-0.2 M NaOH several times.
3. Wash the column with water for 20 column volumes.
4. If problem persists, inject 100-250 µL of 20% acetic acid aqueous solution several times.
5. Wash the column with water for 20 column volumes.

If the issue still persists, replace with a new column.

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