Column Care and Use Instructions
BioPro HIC BF
for Hydrophobic Interaction Chromatography

1. Introduction

Thank you for purchasing a BioPro HIC BF 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 BF columns, which use non-porous polymer beads modified with butyl group, allow for fast and highly efficient separation.

BioPro HIC BF 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 BF
Resin	Hydrophilic non-porous polymer beads
Ligand	Butyl
Particle size	4 μm
Column size	100 mm X 4.6 mm I.D.
Recommended flow rate	0.5 - 1.0 mL/min
Maximum flow rate	1.2 mL/min
Maximum pressure	20 MPa
pH range	2.0 - 12.0
Temperature	10 °C - 60 °C
Column hardware	Stainless steel

3. Column Installation

- BioPro HIC BF columns have Waters-style endfittings.
- 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 BF 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 50%. 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 (XRPRCP02).

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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