Amber HIC chromatography column Instruction Manual

V1.0.2

1. Introduction

The Amber HIC hydrophobic interaction chromatography column is filled with a uniform spherical, highly cross-linked, pore-free polystyrene/divinylbenzene (PS/DVB) material. By uniformly and densely bonding hydrophobic ligands such as ethyl (ethyl), propyl (propyl), butyl (butyl), and phenyl (phenyl) onto the PS/DVB surface, four hydrophobic interaction chromatography columns with varying degrees of hydrophobicity from low to high are obtained: Amber HIC Ethyl, Amber HIC Propyl, Amber HIC Butyl, and Amber HIC Phenyl. The Amber HIC chromatography column is particularly suitable for separating monoclonal antibodies (Mabs), antibody-drug conjugates (ADCs), and related protein fragments with differing hydrophobic properties.

2. Column Inspection and First Use

Upon receiving the chromatographic column, please confirm that the received column matches the information you ordered. Verify that the direction of the mobile phase is printed on the column label and that the column remains undamaged during transportation.

During transportation and storage, solvent drying may occur. In such cases, it is recommended to flush the chromatographic column with 2 column volumes of ultrapure water at a flow rate of 0.2 ml/min, followed by rinsing the activated column with 10-20 column volumes of experimental buffer. Persistent baseline fluctuations may indicate the presence of bubbles within the column. Continue flushing at a higher flow rate for 2-5 minutes.

If the pH value of the mobile phase used in the experiment differs significantly from that of the solvent used for column preservation, it is recommended to flush the chromatographic column with 10 column volumes of the experimental mobile phase prior to each use.

Note: A flow rate of 0.1-1.5 ml/min is recommended.

3. Column connection

The inlet and outlet of the chromatographic column were connected in the direction indicated by the arrow on the column label.

The port section of the chromatographic column connecting tube must be smooth, with no burrs or beveled cuts.

Where possible, the connecting tubing at both ends of the chromatographic column should be as short as possible, and the inner diameter of the connecting tubing should be minimized to prevent sample diffusion from causing situations where the true column efficiency cannot be accurately reflected.

4. Temperature and pH range for use

The chromatographic column is used within a pH range of 2.0 to 12.0.

The maximum operating temperature is 80℃.

5. Column storage

The solvent used for storing Amber HIC chromatography columns at the time of shipment was 25 mmol/L sodium phosphate buffer solution (pH 7.0).

When not in use for an extended period, it is recommended to store the Amber HIC hydrophobic interaction chromatography column in room temperature water or a 25 mmol/L sodium phosphate solution with a pH of 7.0.

6. Application

Since the surface coatings of PS/DVB are chemically bonded, they exhibit extremely high stability. These chromatographic columns are compatible with most buffer salt solutions and organic-water mixed phases, such as ammonium sulfate, sodium acetate, phosphate buffer solutions, methanol, acetonitrile, and tetrahydrofuran-water mixtures. A 25 mmol/L sodium phosphate buffer solution (pH 7.0) is recommended as the mobile phase.

7. Column maintenance

To maximize the lifespan of the chromatographic column, it is recommended to add a guard column between the injector and the column, or an online filter with a pore size of 0.45 μm. High-performance liquid chromatography (HPLC) columns are consumables, and proper operation and maintenance are crucial for extending their lifespan.

(1) Mobile phase

When using buffer saline solution, it must first be filtered through a 0.45 μm membrane prior to use, followed by ultrasonic degassing for 15 minutes.

(2) Samples

Impurities in samples are one of the primary causes of column contamination and reduced column efficiency in chromatography. For complex samples, appropriate pretreatment methods should be employed to remove impurities, followed by filtration using 0.22μm or 0.45μm filter membranes prior to injection. If sample processing is impractical, the use of protective columns is recommended. In some cases, incompatibility between sample solvents and mobile phases (e.g., immiscibility or polarity differences) may lead to poor chromatographic peak resolution or the appearance of ghost peaks. It is advisable to dissolve samples using the mobile phase whenever possible.

8. Common Issues and Solutions During Use

During use, in certain special circumstances, there may be instances of low sensitivity for certain substances to be tested.

Solution: Adding some organic solvents as modifiers to the mobile phase can enhance the detection sensitivity of certain substances.

Note: The content of organic solvents should generally not exceed 30%. Pay attention to the salting-out phenomenon when adding organic solvents to the buffer salt mobile phase.

Scan the QR code for more product information.

Suzhou Elite Science & Technology Co., Ltd.
Address: 701#, Building 10, Liando U Valley, No. 179 Zhujiawan Street, Gusu District, Suzhou, Jiangsu, China.
Tel: +86-512-67997535
Fax: +86-512-67997535

Elite (Dalian) Analytical Instruments Co., Ltd.
Address: No. 2-2 Xuezi Street, Qixianling Subdistrict, Dalian High-Tech Industrial Zone, Liaoning, China.
Tel: +86-0411-84753333
Fax: +86-0411-84753333

Customer service email : info@eliteHPLC.com

http://www.eliteHPLC.com