Amber IEC chromatography column Instruction Manual

V1.0.2

1. Introduction

The packing material of Amber ion exchange (IEC) chromatography column consists of a core of highly cross-linked polystyrene/divinylbenzene (PS/DVB) resin, with a dense nanometer-thickness hydrophilic coating bonded to its surface. An additional layer of uniformly distributed ion exchange functional groups is bonded onto the surface of the hydrophilic layer.

Amber ion exchange stationary phases include four types: strong cation exchange (SCX), weak cation exchange (WCX), strong anion exchange (SAX), and weak anion exchange (WAX). Amber SCX, WCX, SAX, and WAX are formed by chemically bonding sulfonic acid groups, carboxyl groups, quaternary ammonium groups, and tertiary amine groups onto the hydrophilic coating surface of non-porous PS/DVB resin. The particle size of the non-porous filler is 5 μm, and the IEC chromatographic column can be used for the separation of proteins, oligonucleotides, polypeptides, polysaccharides, cell lysates, nanoparticles, and nanotubes.

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.

Amber SAX and WAX chromatography columns were stored in 20 mM Tris buffer solution (pH 8.0), while SCX and WCX chromatography columns were stored in 20 mM phosphate buffer solution (pH 6.0). Upon receiving the chromatography columns, it is recommended to flush and activate them with 10–20 column volumes of storage solvent. Subsequently, evaluate the new columns to verify their performance and document the evaluation results.

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°C. To prolong the service life of the chromatographic column, it is recommended to operate within the range of 10–50°C. Prolonged operation at temperatures exceeding 80°C may damage the column, with particularly noticeable effects under high pH conditions (greater than 12 or less than 2). Avoid sudden temperature increases during use.

5. Column storage

For long-term storage, Amber SAX and WAX chromatographic columns should be preserved in 20 mM Tris buffer solution (pH 8.0) containing 0.1% NaN3*, while SCX and WCX columns should be stored in 20 mM phosphate buffer solution (pH 6.0) containing 0.1% NaN_3*. Prior to storage, ensure the plugs are tightly sealed against the column heads to prevent column bed drying. The optimal storage temperature is room temperature.

*Note: *, Due to the high toxicity of sodium azide, safety precautions must be taken during use, and protective measures should be implemented. Alternatively, other bacteriostatic agents with equivalent efficacy may be employed, such as the commercial bacteriostatic agent ProClin 300.*

6. Application

Since the surface modification of PS/DVB is entirely based on chemical bonding, Amber ion exchange columns exhibit excellent stability. This stationary phase is compatible with most buffers such as ammonium acetate, hydrochloride, and Tris. Amber SCX, Amber WCX, Amber SAX, and Amber WAX resins are based on pore-free PS/DVB particles, with hydrophilic layers covering their surfaces and uniformly bonded ion exchange functional groups. These stationary phases possess three key characteristics: First, the nanoscale hydrophilic layer completely eliminates non-specific interactions between the carrier and biological samples; Second, the pore-free particle structure minimizes lateral sample diffusion while inhibiting internal diffusion into the filler particles; Third, proprietary techniques enable the synthesis of homogeneous and dense ion exchange layers. The unique Amber ion exchange chromatographic stationary phase provides optimal resolution and separation efficiency for proteins, oligonucleotides, carbohydrates, and polypeptides.

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 0.22 μm or 0.45 μm. High-performance liquid chromatography (HPLC) columns are consumables, and proper operation and maintenance are crucial for extending their service life.

(1) Mobile phase

The organic solvent used in the mobile phase must be chromatographically pure. For the mobile phase water, ultrapure water or double-distilled water from glassware is preferred. Buffer salts must be filtered through a solvent filter (0.45 μm membrane) before use. The mobile phase should be prepared immediately upon use, as prolonged storage of saline solutions may lead to bacterial growth or precipitation.

SAX and WAX columns in ion exchange chromatography columns are compatible with nonionic and amphoteric ion detergents, but incompatible with anionic detergents. SCX and WCX columns are compatible with nonionic and amphoteric ion detergents, but incompatible with cationic detergents.

(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 0.22 μm or 0.45 μm 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

After repeated use, certain samples may adsorb onto the inlet sieve plate or packing material. When accumulation reaches a certain level, pressure elevation accompanied by peak broadening will occur, necessitating column cleaning. The standard procedure involves: disconnecting the detector, reversing the column orientation, and flushing the column with a cleaning solution at 10-15 column volumes below 50% of the maximum recommended flow rate.

In common cleaning solutions, low-pH salt solutions are effective for removing alkaline proteins, while high-pH salt solutions facilitate the removal of acidic proteins, and organic solvents can eliminate hydrophobic proteins. The Amber anion exchange column utilizes a 75% acetonitrile solution containing 150 mM potassium nitrate (pH adjusted to 2.0 with HCl), whereas the Amber cation exchange column employs a 50 mM phosphate buffer solution containing 1.0 M NaCl (pH 10).

When the aforementioned methods fail to resolve the issue, it is recommended to replace the chromatographic column with a new one.

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