Amber SEC chromatography column Instruction Manual

V1.0.2

1. Scope of application

The Amber SEC 5μm, Amber SEC 5μm Plus, Amber SEC 3μm, and Amber SEC 3μm Plus series size exclusion chromatography columns all utilize high-purity silica gel with special surface modifications as packing materials. The modification involves chemically bonding a homogeneous, hydrophilic, nanometer-thickness neutral polymer film onto the silica gel surface. These columns can meet diverse customer requirements for resolution and column efficiency, providing a stable, reproducible, and high-resolution optimal solution for biomolecular size exclusion chromatography. Packing material pore size and protein separation molecular weight range:

100 Å: 100–100,000
150 Å：500 ~ 150,000
300 Å：5,000 ~ 1,250,000
500 Å：15,000 ~ 5,000,000
1000 Å：50,000 ~ 7,500,000
2000 Å：>10,000,000

2. Performance Acceptance

Upon receipt of the chromatographic column, perform unpacking inspection and verification. Subsequently, conduct testing according to the chromatographic conditions specified in the column evaluation report to confirm column quality.

sample ：

① Thyroglobulin (1.0 g/mL)
② Bovine serum albumin (1.0 g/mL)
③ Ribonuclease A (1.0 g/mL)
④ Urine (0.1 g/mL)

All samples were dissolved and diluted using the mobile phase.

Note: In case of any discrepancy between the above conditions and the chromatographic column detection report, the detection report shall prevail.

3. Chromatographic 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 recommended operating temperature should not exceed 80°C.

The optimal operating temperature with the longest lifespan ranges from 10 to 30°C. Continuous use at higher temperatures (>80°C) may cause damage to the chromatographic column.

Avoid sudden temperature increases or decreases during use.

To maintain optimal performance and maximum lifespan, use a pH range of 2-8.5.

5. Chromatographic column storage

When the column is not in use for an extended period, it should be stored in water containing 0.02% sodium azide or 20% ethanol. Each chromatographic column is equipped with two detachable plugs. To prevent column bed drying, seal both ends of the column with the provided plugs during storage, and 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 used, such as the commercial bacteriostatic agent ProClin 300.

6. Chromatographic column maintenance

High-performance liquid chromatography (HPLC) columns are consumables, and standardized operation and maintenance are crucial for extending their lifespan.

(1) Precautions before using the chromatographic column

Unless otherwise specified, the chromatographic column storage solutions produced by our company are all the mobile phases indicated in the evaluation reports. Before use, users must ensure that the mobile phase specified for chromatographic column evaluation is compatible with the actual mobile phase used for sample analysis.

(2) Mobile phase

To prevent column clogging, all solvents including buffer solutions must be filtered through 0.45 μm or 0.2 μm filter membranes prior to use. The mobile phase should be prepared immediately upon use, as prolonged storage of saline solutions may lead to bacterial growth or precipitation. The Amber SEC 5μm column can employ water or organic solvent-water mixtures (e.g., methanol or acetonitrile aqueous solutions) as mobile phases. The mobile phase must be degassed before use.

(3) 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.

7. Column Maintenance

Some samples may be adsorbed onto the inlet sieve plate or packing material. When adsorption accumulates to a certain extent, it typically manifests as increased back pressure and broadened peak shape. In such cases, chromatographic column cleaning is required.

Selection of cleaning agent:

(1) Low-pH high-salt solution (e.g., 0.5 M Na_2SO_4, pH 3.0).

(2) Water-soluble organic solvents (e.g., 10%-20% methanol/acetonitrile/ethanol) were mixed with buffer solutions (e.g., 50 mM sodium phosphate, pH 7.0).

Note: *Use of corrosive solvents such as NaOH is prohibited.

The general procedure for column cleaning is as follows:

(1) Disconnect the chromatographic column from the detector.
(2) Reverse the chromatographic column and perform flushing.
(3) Rinse the chromatographic column at flow rates below 50% of the maximum recommended flow rate. Monitor column pressure changes. If column pressure significantly exceeds normal levels, reduce the flow rate or switch to a low-viscosity buffer solution as the eluent.
(4) Generally, rinsing with a cleaning solution at 10-15 column volumes is sufficient. When changing the solution, rinse the chromatographic column thoroughly with 3-5 column volumes of distilled deionized water.
If improper pH value is used, it is usually difficult to restore the chromatographic column.

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