

# Amber Protein A chromatography column

## Instruction Manual

V1.0.2

### 1. Introduction

The Amber Protein A Chromatography Column is specifically designed for HPLC high-performance liquid chromatography columns. Its stationary phase utilizes porous polystyrene divinylbenzene (PS/DVB) with an average particle size of 20  $\mu\text{m}$  as the matrix, coated with a hydrophilic layer on the surface. Recombinant protein A is chemically bonded onto this coating, enabling binding of immunoglobulins containing Fc fragments except IgG3. This column can be used for the quantitative analysis and small-scale purification of antibodies and fusion proteins (which specifically bind to recombinant protein A).

### 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.

When using a chromatographic column for the first time, flush the column with 5-10 column volumes of elution buffer to remove the storage solvent. Then balance the column with 10-15 column volumes of initial/dilution buffer. A pre-column filter (0.5  $\mu\text{m}$ ) must be used to minimize column contamination.

### 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 of the chromatographic column cannot be accurately reflected.

### 4. Temperature and pH range for use

The recommended operating temperature ranges from 2°C to 40°C, and freezing is prohibited.

To maintain optimal performance and maximum lifespan, use a pH range of 2-10.

### 5. Column storage

If not used for an extended period, store the Amber Protein A chromatography column under the following conditions:

- Neutral pH solution containing bacteriostatic agents\* (e.g., 0.02% sodium azide).
- Keep in the refrigerator, but do not freeze!
- To prevent column bed drying, secure both ends of the chromatographic column with plugs. Drying may lead to reduced column efficiency.

**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. Column regeneration

Amber Protein A chromatography columns are generally highly stable. When reusing the column, monitor column pressure and perform a standard sample test. If pressure increases or sample recovery rate changes, cleaning is required to remove residual substances from the column sieve plate and packing material. Suitable cleaning solutions for Protein A columns include: 2-6 mol/L guanidine hydrochloride, 1 mol/L acetic acid, 20% ethanol, 1 mol/L acetic acid plus 20% ethanol, 20% isopropanol, elution buffer with pH 1.5-2.0, and elution buffer containing 1-2 mol/L sodium chloride.

### 7. Column maintenance

High-performance liquid chromatography (HPLC) columns are consumables, and proper 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

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  $\mu\text{m}$  membrane) prior to use. The mobile phase should be prepared immediately before use, as prolonged storage of saline solutions may lead to bacterial growth or precipitation.

#### (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 0.22 $\mu\text{m}$  or 0.45 $\mu\text{m}$  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

To ensure effective sample binding and prevent contamination of the chromatographic column sieve plate, samples are typically prepared as follows:

- Dissolve the sample into or replace the solvent with the initial/dilution buffer, which is particularly important for large-volume samples (exceeding 25% of column volume).
- Centrifuge or filter samples prior to injection (0.22 or 0.45  $\mu\text{m}$ ).
- Heat treatment of serum samples (incubated at 56°C for 30 minutes) to remove fibrinogen that may clog the chromatographic column.
- Perform degreasing treatment on the sample, as lipids can

cause irreversible contamination of the chromatographic column.  
(e) All samples should be filtered using 0.45  $\mu\text{m}$  or 0.2  $\mu\text{m}$  filter membranes prior to use.

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](mailto:info@eliteHPLC.com)  
<http://www.eliteHPLC.com>