TK-Col Benzamidine 4FF

TK-Col 16/10 Benzamidine 4FF

TK-Col 26/10 Benzamidine 4FF

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TA-Benzamidine 4FF (HS) is an affinity chromatography medium made by coupling p-aminobenzenecarboximidamide to agarose gel TA-4FF, which is commonly used for the isolation and purification of serine proteases or the removal of serine proteases from biological samples. Benzamidine analogs are broad-spectrum inhibitors of serine proteases (e.g., trypsin, thrombin, urokinase, kinin-releasing enzyme, and prokinin-releasing enzyme), and can be used as ligands for the purification of such substances.

TK-Col Benzamidine 4FF pre-packed columns are ready-to-use affinity chromatography columns filled with TA-Benzamidine 4FF media in TK-EC 1ml ,TK-EC 4.9 ml ,TK-EC 5ml ,TK-EC 20ml chromatographic vacutainer columns. TK-Col 16/10 Benzamidine 4FF pre-packed columns are ready-to-use affinity chromatography columns filled with TA-Benzamidine 4FF medium in TK-EC 16/20 columns; TK-Col 26/10 Benzamidine 4FF pre-packed columns are ready-to-use affinity chromatography columns filled with TA-Benzamidine 4FF medium in TK-EC 26/20 columns. This series of columns eliminates the hassle of loading the columns by the customer and the risk of poor column performance. This type of pre-packed columns is widely used in laboratory process development, small amount preparation of samples, and is suitable for the separation and purification of biomolecules such as trypsin. It has the following features:

- Ready-to-use
- Volume stabilization of the column bed
- Good physical and chemical resistance

2. Technical parameters

Table 1 TA-Benzamidine 4FF (HS) technical parameters

Appearance	White slurry, layered on placement
Base Frame	4% highly cross-linked agarose
Average particle size	90μm
Functional groups	p-Aminobenzamidine
Ligand density	≥12μmol/mL filler
Binding load	≥35mg trypsin/mL filler
Pressure resistance	0.3 MPa
Chemical stability	Common aqueous solutions: 8M urea, 6M guanidine hydrochloride, pH=1,2,3,4 Hydrochloric acid solution, 0.025M borax solution pH=8, 9, 10, 11
pH stability	2~8 (working); 1~9 (CIP)
Storage	2~8°C, 20% ethanol or 2% benzyl alcohol, 50 mM NaAc pH 4.0
Recommended Flow Rate	30-300cm/h

Table 2: Technical parameters for each pre-assembled column (see end page for item number)

Product name	Prepacked resin	Prepacked column volume ml	Inner diameter× Column bed height mm×mm	Recommended flow rate + ml/min	Storage	Pressure resistance	Sieve plate aperture (μm)
TK-Col Benzamidine 4FF	TA-Benzamidine 4FF	1	7×25	0.2-2.0	2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments).	0.3MPa（3bar）	10
		4.9	8×100	0.2-2.5			
		5	16×25	1.0-10.0			
		20	16×100	1.0-10.0			
TK-Col 16/10 Benzamidine 4FF		19.1-21.1	16×100（±5）	2.0-10.0			
TK-Col 26/10 Benzamidine 4FF		50.4-55.7	26×100（±5）	<26			

3. Methods of use

- *TK-Col 16&26 series chromatography columns are made of glass and should be handled gently to prevent breaking or affecting the column efficiency.*
- *To avoid clogging the column, all samples and buffers need to be filtered through 0.45um membrane.*
- *In order to get a good separation effect, avoid too much temperature difference between the buffer and the column.*
- *Keep the column out of direct sunlight.*
- *Chromatography columns can be used in a chromatography cooler, but the flow rate needs to be reduced appropriately.*

3.1 Connecting the column to the chromatography system

- Open the package and take out the column
- Check whether the column is intact, and whether the column has been dried out during transportation, if any of the above situations occurs, please contact Chutian Microsphere sales representative in time.
- Fix the column next to the chromatography system and pay attention to the flow direction of the column.
- Start the chromatography system, make sure the air bubbles in the chromatography system are drained, and set the alarm pressure of chromatography system to 0.3MPa, then adjust and keep the flow rate running at 0.2ml/min.
- After the chromatography system is purged of air bubbles, open the upper and lower plugs of the chromatography column and connect the chromatography column under low flow rate operation.

3.2 Pretreatment of chromatography columns

- Rinse, the chromatography column is stored in 20% ethanol or 2% benzyl alcohol (for international transportation) during transportation, first rinse off the storage solution with 2 column volumes of distilled water.
- Sterilization, for sample safety, it is recommended to rinse 2 column volumes with 0.5M NaOH before rinsing 2 column volumes with distilled water for the first use.

3.3 Equilibration of Chromatographic Columns

- Binding buffer: Generally use neutral buffer, such as 50mM Tris, 0.5M NaCl, pH 7.4.
- Use the recommended flow rate with equilibration buffer to flush the chromatographic column, to be exported to the pH and conductivity of the buffer and the buffer before entering the chromatographic column that is to say that the chromatographic column equilibrium is good, generally need 2~5 column volume.

3.4 Flow rate

- Depending on the type of chromatography column, flow rates within the recommended flow rate range are generally selected, with slower flow rates for higher column heights. (See Table 2)

3.5 Sampling

- The viscosity of the sample needs to be appropriate, high viscosity samples will cause irregular flow rates during the chromatography process.
- In order to avoid clogging the chromatographic column will reduce the resolution efficiency and service life of the column, so the sample solution needs to be centrifuged or filtered with a 0.45 μm filter before the sample is loaded. The pH and conductivity of the sample were adjusted to match that of the equilibration buffer, and the volume of the sample was determined according to the impurity content in the sample and the binding load of TA-Benzamidine 4FF.

3.6 Rinse

- Rinse with equilibration buffer until the UV absorption value drops to the appropriate value.

3.7 Eluent

- Can be elution mode 1: commonly used to lower the pH for elution, such as: 50mM glycine, pH 3.0. The collected elution is immediately neutralized with 1M Tris, pH 9, and 60~200 μl 1M Tris is required for 1ml elution.
- Elution method 2: Optionally, competitive elution with the addition of p-aminobenzenecarboxamidine is also available, e.g., 50 mM Tris, 0.5 M NaCl, 20 mM p-aminobenzenecarboxamidine, pH 7.4.

3.8 Regeneration and rebalancing

- Regeneration: Rinse the column with purified water or 30% isopropanol (70% ethanol).
- Re-equilibration: after rinsing with equilibration buffer the column is ready for the second sample and so on.

3.9 Column Effectiveness Evaluation

Column efficiency can be determined by using acetone as indicator or NaCl as indicator, and the indicator solution and mobile phase are prepared according to the following table.

Table 3: Column efficiency determination methods

Methods	Acetone Method for Column Efficacy	Column Efficacy by NaCl Method
Sample	1.0% (v/v) acetone in water	0.8M NaCl (dissolved in water)
Sample volume	1.0% column volume	1.0% column volume
Mobile phase	Water	0.4M NaCl aqueous solution
Flow rate	30 cm/h	30 cm/h
Detection Data	UV 280 nm	Conductivity

3.10 Calculating Column Effect

Theoretical plate height (HETP), theoretical number of plates (N) and asymmetry factor (As) were calculated from the UV or conductivity curves with the following equations:

HETP=L/N

$N=5.54(V_R/W_h)^2$

Where: V_R = retained volume

W_h=half peak width

L=column height

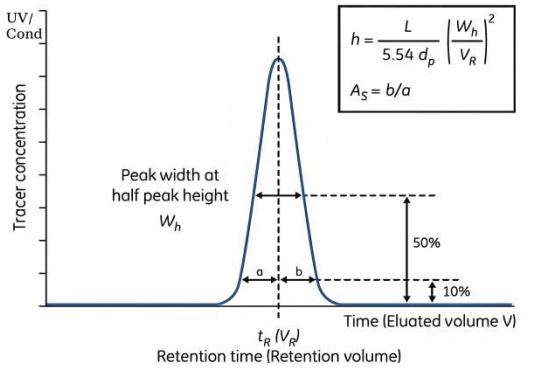
N=theoretical plate number

The units of V_R and W_h should be the same;

As=b/a

Where: a= first half peak width at 10% peak height

b= second half peak width at 10% peak height



3.11 Evaluation of results

$h=HETP/d_{50v}$

d_{50v} = median particle size volume distribution (cm)

The h-value calculated by the above formula is less than 3, and the asymmetry factor is 0.8~1.8 then it is judged to be qualified. For unsatisfactory column efficiency the reason needs to be analyzed and the column reloaded.

4. Cleaning and regeneration

As the number of times the chromatography medium is used increases, the accumulation of contaminants on the chromatography column also increases. Regular in-situ cleaning prevents the accumulation of contaminants and maintains a stable working condition.Customers can determine the frequency of in-situ cleaning according to the degree of media contamination during use (if contamination is severe, it is recommended that in-situ cleaning be performed after each use to ensure reproducible results).

Regeneration: 2 column volumes of high pH buffer (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low pH buffer (0.1 M sodium acetate, 0.5 M NaCl, pH 3) were washed alternately three times; 10 column volumes of binding buffer equilibrated the chromatography column.

- If the medium is used for a period of time and the protein binding capacity decreases due to excessive impurities deposited on the surface, the medium needs to be cleaned with the following steps:
- Precipitated or denatured material cleaning:
  - was washed with 2 column volumes of 6 M guanidine hydrochloride followed by 5 column volumes of equilibration buffer;
- Cleaning of hydrophobic bonding substances:
  - Wash with 2 column volumes of 70% ethanol followed by 5 column volumes of equilibration buffer

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol (pH 4.0) preservation solution containing 50 mM sodium acetate is not bacteriostatic or pyrogenic, it is recommended that TA-Benzamidine 4FF(HS) media can be treated with 20% ethanol containing 0.1 mM acetic acid for more than 12 h prior to and during use to minimize the risk of microbial contamination.

TA-Benzamidine 4FF(HS) is sold with 20% ethanol containing 50 mM sodium acetate or 2% benzyl alcohol (pH 4.0) as a preservation solution. After use, TA-Benzamidine 4FF(HS) should be stored in a 20% ethanol (pH 4.0) solution containing 50 mM sodium acetate and kept at 2-8°C in an airtight container. To prevent ethanol evaporation and microbial growth, it is recommended that the preservation solution be replaced with a fresh one every 3 months.

6. Destruction and recycling

Since TA-Benzamidine 4FF(HS) is difficult to degrade in nature, incineration is recommended for environmental protection.

7. Ordering Information

Table 4 Article number and packaging

Product	Item No.	Norm
TK-Col Benzamidine 4FF	Y6315	1×1ml
	Y6317	5×1ml
	Y6316	1×5ml
	Y6318	5×5ml
	Y631803	1×4.9ml
	Y631804	1×20ml
TK-Col 16/10 Benzamidine 4FF	Y6319	1pac.
TK-Col 26/10 Benzamidine 4FF	Y6320	1pac.