TK-Col Heparin HP

TK-Col 16/10 Heparin HP

TK-Col 26/10 Heparin HP

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TA-Heparin HP is a race-specific affinity chromatography medium made by covalently coupling heparin to high cross-linking agarose. It is widely used for the separation and purification of various biomolecules, especially enzymes, including antithrombin III, thrombin-like enzymes, human coagulation factors IX, XI and VIII, lipoprotein lipases, collagenases and DNA polymerases, and it can also be used for the separation and purification of human interleukin, human prostate growth factor, recombinant human vascular endothelial growth factor, chondrocyte growth factor, basic fibroblast growth factor, recombinant human acidic fibroblast growth factor, recombinant hepatocyte growth factor, recombinant murine cofactor II, recombinant human platelet factor IV, recombinant human endothelial inhibitor and recombinant human keratinocyte growth factor, etc. The medium is characterized by physical and chemical stability, non-shedding of ligands, long service life, wide range of applications and easy scale-up.

TK-Col Heparin HP pre-packed columns are ready-to-use affinity chromatography columns filled with TA-Heparin HP media in TK-EC 1 ml ,TK-EC 4.9 ml ,TK-EC 5 ml ,TK-EC 20 ml chromatography vacutainer columns. TK-Col 16/10 Heparin HP pre-packed columns are ready-to-use affinity columns filled with TA-Heparin HP media in TK-EC 16/20 chromatographic vacutainers. TK-Col 26/10 Heparin HP pre-packed columns are ready-to-use affinity columns filled with TA-Heparin HP media in TK-EC 26/20 chromatographic vacutainers. This series of columns eliminates the hassle of loading the columns by the customer and the risk of poor column performance. These pre-packed columns are widely used for laboratory process development, small amount sample preparation, and are suitable for the separation and purification of biomolecules such as thrombin-like enzymes. 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-Heparin HP technical parameters

Appearance	White slurry, layered on placement
Base Frame	Highly cross-linked 6% agarose
Average particle size	34μm (24~44μm)
Functional groups	Heparin
Ligand density	~10mg Heparin ligand/mL medium
Chemical Stability	Stable in all commonly used water-soluble buffers: 8M urea, 6M guanidine hydrochloride, 70% ethanol, 50mM sodium acetate (pH4), 10% glycerol, 0.1M NaOH (20°C for one week)
pH Stability	5~10
Temperature resistance	Use temperature 2~30℃, can not be frozen, can be 121℃ autoclaved for 30min (high pressure with 20mM NaH2PO4, pH7.5 cycle)
Pressure resistance	0.3MPa
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol, 50mM sodium acetate
Recommended Flow Rate	60~300cm/h

+2% benzyl alcohol for foreign shipments only or as specified by the customer

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 Heparin HP	TA-Heparin HP	1	7×25	0.2-1.0	2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments).	0.3MPa（3bar）	10
		4.9	8×100	0.2-1.0			
		5	16×25	1.0-5.0			
		20	16×100	1.0-5.0			
TK-Col 16/10 Heparin HP		19.1-21.1	16×100（±5）	2.0-5.0			
TK-Col 26/10 Heparin HP		50.4-55.7	26×100（±5）	<13			

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: 20-50mM PB or Tris, pH7.4-8.0, 0.15M NaCl can be added to inhibit non-specific adsorption.
- Elution buffer: 20-50 mM PB or Tris, 1-2 M NaCl, pH 7.4-8.0, NaCl concentration needs to be adjusted appropriately according to the binding strength of the target protein
- Use the recommended flow rate to flush the chromatographic column with equilibration buffer, the pH and conductivity of the buffer to be exported are consistent with that of the buffer before entering the chromatographic column that is to say that the chromatographic column is well equilibrated, and it generally requires 2-5 column volumes.

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 should be adjusted to match that of the equilibration buffer, and the volume of the sample should be determined according to the impurity content in the sample and the binding load of TA-Heparin HP.

3.6 Rinse

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

3.7 Eluent

- When optimizing the elution conditions, a linear gradient or step gradient can be used to increase the elution strength in the eluent, to elute substances with different binding strengths from the chromatography column, to collect different fractions, and to detect where the target is located.

3.8 Regeneration and rebalancing

- Regeneration: rinse the column with a salt containing a high concentration (e.g. 2M NaCl).
- 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

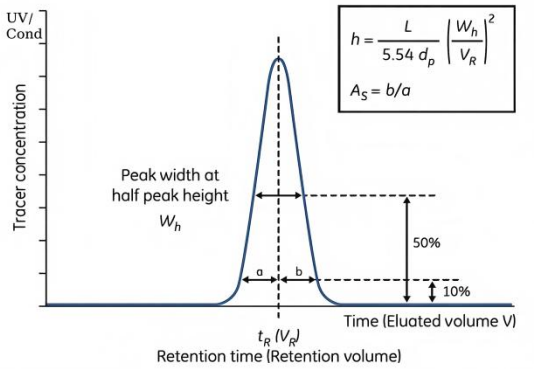
N=theoretical plate number

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).

Recommended cleaning conditions for different types of impurities and contaminants are as follows:

- **Removal of ion-bonded proteins:** wash with 3-5 column volumes of 2M NaCl followed by 3-5 column volumes of pure water.
- **Removal of precipitated or denatured proteins:** Wash with 2 column volumes of 0.1M NaOH, followed by 5-10 column volumes of pure water, or 6M guanidine hydrochloride or 8M urea.
- **Removal of hydrophobically bound proteins from:** 0.1-0.5% nonionic detergent wash followed by 3-5 column volumes of pure water.

Note: The flow rate can be selected from 30-60cm/h during bit cleaning, and reverse cleaning is recommended.

For immediate use after cleaning, rinse 3-5 column volumes with equilibration buffer and use, or if not to be used for a short period of time, wash 3-5 column volumes with 20% ethanol solution and store them.

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution containing 50mM sodium acetate is not bactericidal or pyrogenic, it is recommended that TA-Heparin HP can be treated with 70% ethanol for more than 12h before and during use to reduce the risk of microbial contamination.

TA-Heparin HP is sold in 20% ethanol or 2% benzyl alcohol containing 50 mM sodium acetate as a preservation solution. After use, TA-Heparin HP should be stored in 20% ethanol containing 50 mM sodium acetate, airtight at 2-30° C. To prevent ethanol evaporation and microbial growth, it is recommended that the preservative be replaced with a fresh solution every 3 months.

6. Destruction and recycling

Since TA-Heparin HP 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 Heparin HP	Y6309	1×1ml
	Y6311	5×1ml
	Y6310	1×5ml
	Y6312	5×5ml
	Y631203	1×4.9ml
	Y631204	1×20ml
TK-Col 16/10 Heparin HP	Y6313	1pac.
TK-Col 26/10 Heparin HP	Y6314	1pac.