TK-Col Plasmid Fine

TK-Col 16/10 Plasmid Fine

TK-Col 26/10 Plasmid Fine

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TH-Plasmid Fine is a thiophilic affinity medium made by immobilizing the sulfur-containing compound 2-mercaptopyridine on fine-grained, highly rigid agarose, with optimized ligand density and suitable affinity for superhelical DNA, and fine-grained microspheres that can enhance the loading of superhelical DNA with higher molecular weight. Thiophilic affinity is based on the principle of separating and purifying biomolecules by utilizing the interaction between electron donors and electron acceptors, which is strengthened in high-salt environments and weakened in low-salt environments.

TK-Col Plasmid Fine preloaded columns are ready-to-use affinity chromatography columns filled with TH-Plasmid Fine 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 Plasmid Fine preloaded columns are ready-to-use affinity chromatography columns filled with TH-Plasmid Fine media in TK-EC 16/20 chromatography vacutainer columns; TK-Col 26/10 Plasmid Fine preloaded columns are ready-to-use affinity chromatography columns filled with TH-Plasmid Fine media in TK-EC 26/20 chromatography vacutainer 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 preloaded 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 superhelical DNA. It has the following features:

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

2. Technical parameters

Table 1 Technical parameters of TH-Plasmid Fine

Appearance	White paste, layered on placement
Base Frame	Highly rigid agarose
Average particle size	34μm (24~44μm)
Functional groups	2-Mercaptopyridine
Ligand density	~3.5mg 2-mercaptopyridine/mL media
Binding load	>2mg superhelical plasmid/mL medium
Chemical Stability	Common aqueous phase solutions: 30% isopropanol, 70% ethanol, 1M acetic acid, 0.1M NaOH
Pressure resistance	0.5MPa
pH stability	3-11 (working), 2-13 (CIP, short-term)
Temperature Stability	Use temperature 2-30°C, can not be frozen.
Storage	2-30°C, 20% ethanol or 2% benzyl alcohol

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 Plasmid Fine	**TH-Plasmid Fine**	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	1.0-4.0			
		5	16×25	1.0-10.0			
		20	16×100	1.0-10.0			
TK-Col 16/10 Plasmid Fine		19.1-21.1	16×100（±5）	2.0-10.0			
TK-Col 26/10 Plasmid Fine		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

- Use the recommended flow rate to flush the chromatography column with binding buffer. The pH and conductance of the buffer to be exported are consistent with that of the buffer prior to entering the chromatography column, which means that the column is equilibrated, and generally 2~5 column volumes are needed.
- Binding buffer: 0.1M Tris, 10mM EDTA, 2.1M (NH4)2SO4, pH7.5.
- Elution buffer: 0.1M Tris, 10mM EDTA, 2.0M (NH4)2SO4, pH7.5

3.4 Flow rate

- According to the type of the chromatographic column, the general selection of flow rate within the recommended flow rate range, for the height of the column in the 10~15cm high case of flow rate: according to the height of the column is generally selected 90~180cm/h flow rate, the higher the height

of the column the slower the flow rate, the higher the height of the column the slower the flow rate. (See table 2)

3.5 Sampling

- Sample and uploading volume: In order to prevent the sample from clogging the column, the sample needs to be filtered with 0.45 μ m microporous filter membrane before uploading, and the pH and conductivity of the sample should be adjusted to be consistent with the equilibrium buffer, the concentration of potassium sulphate, etc. affects the binding load.
- Determine the up-sampling volume according to the load of the medium and the concentration of superhelical DNA in the sample, generally according to 80% of the load up-sampling.

3.6 Rinse

- Rinse with wash buffer until the UV absorption value drops to the appropriate value. Rinse off the ring-opening plasmid DNA using the recommended buffer.

3.7 Eluent

The recommended elution buffer can be used to elute and collect the elution peaks.

Elution buffer: 0.1 M Tris, 10 mM EDTA, 1.7 M (NH4)2SO4, 0.3 M NaCl, pH 7.5

3.8 Regeneration and rebalancing

Regeneration: Wash buffer to rinse the chromatography column. Wash buffer: 20 mM PB, 30% isopropanol, pH 7.5

Re-equilibration: Equilibrate the column with binding buffer and set aside.

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 4: 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

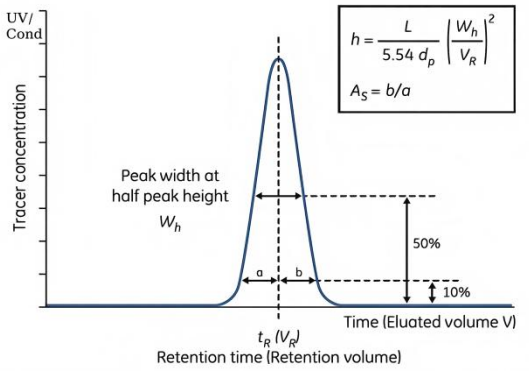
3.10 Calculating Column Effect

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 can effectively prevent the accumulation of contaminants and maintain the stable working condition of the chromatography medium.Customers can determine the frequency of in-situ cleaning according to the degree of contamination of the media during use (if the contamination is more serious, it is recommended to carry out in-situ cleaning after each use to ensure the reproducibility of the results).

Denatured proteins: Use 0.5M NaOH to wash 2-4 column volumes, and equilibrate with 2~4 column volumes of equilibration buffer after NaOH washing with purified water.

Strong hydrophobic substances or lipids: Use 2-4 column volumes of 20 mM PB, 30% isopropanol, pH 7.5 buffer to rinse the column, and use purified water to wash before and after rinsing.

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solutions are not bactericidal or pyrogenic, it is recommended that TH-Plasmid Fine media can be treated with 70% ethanol for 12h before and during use to reduce the risk of microbial contamination.

TH-Plasmid Fine are sold with 20% ethanol or 2% benzyl alcohol as preservation solution. After use, TH-Plasmid Fine should be stored in 20% ethanol at 2~30°C in airtight storage. To prevent ethanol volatilization and microbial growth, it is recommended that the preservation solution be replaced with fresh preservation solution every 3 months.

6. Destruction and recycling

Since TH-Plasmid Fine is difficult to degrade in nature, incineration is recommended to protect the environment.

7. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TK-Col Plasmid Fine	Y6255	1×1ml
	Y6257	5×1ml
	Y6256	1×5ml
	Y6258	5×5ml
	Y625803	1×4.9ml
	Y625804	1×20ml
TK-Col 16/10 Plasmid Fine	Y6259	1pac.
TK-Col 26/10 Plasmid Fine	Y6260	1pac.