TH-Plasmid Fine Affinity Chromatography Resin

Product Manual

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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.

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℃, can not be frozen.
Storage	2-30℃, 20% ethanol or 2% benzyl alcohol

2. Methods of use

2.1 Chromatography column loading

Note: It is best to equilibrate the media suspension to room temperature before loading the column.

- Calculate the amount of TH-Plasmid Fine needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.10 (i.e., compression ratio of approximately 1.10)

  Volume of media suspension required = volume of settling media ÷ concentration of media suspension.

  Note: The concentration of Truking Microsphere's original packaging media suspension is 80%, for non-original concentration of media suspension, customers can calculate the required volume according to the actual concentration of media suspension.

- Media washing: Shake the media suspension well and measure the volume calculated by the above method, pour it into a funnel, draw off the liquid and wash it with about 3mL of filling solution (20% ethanol+0.2M

NaCl)/mL of media, repeat the washing 3 times, each time when adding the washing solution, it is necessary to stir it with a glass rod or stirring stick, so as to better wash off the original preservation solution.

- Preparation of Column Mounting Gel Suspension: Transfer the cleaned medium from the funnel to a beaker or other suitable container, add the column mounting solution until the concentration of the gel suspension is 50~75%, stir well and set aside.
- Take a cleaned TK-EC chromatography column (the diameter of TK-EC series chromatography columns ranges from 1cm to 45cm in various specifications to meet the different sizes of chromatography applications), drain the membrane air bubbles at the bottom of the column and keep about 1cm high liquid column at the bottom of the column, and adjust the column so that it is perpendicular to the ground.
- Pour the stirred gel suspension slowly into the chromatography column one at a time, taking care not to bring in air bubbles.
- Connect the upper column head to the chromatography system or peristaltic pump, drain the air bubbles under the screen of the upper column head (for the chromatography column with diameter less than 20cm, you can use the peristaltic pump or the earwash ball to suck out the air bubbles under the screen after turning the column head upwards), put the column head into the chromatography column, shake the column head so that the air bubbles can be discharged from the edge of the column head, and then screw the sealing knob tightly. (For the chromatography column with diameter >30cm, do not tighten the sealing ring too much, press down the head of the shaft to let the liquid inside the column back out through the column head to discharge the air bubbles inside the column head, and then tighten the sealing knob).
- Set the flow rate (for the loading column height of 15~20cm TH-Plasmid Fine can be set to 300cm/h), start the pump pressure until the glue surface is stable, if the pressure is more than 0.3MPa during the loading process, you need to reduce the flow rate appropriately. Mark the height of the column bed when it is stable.
- Remove the column loader (if there is one), lower the column head to the position of about 0.5cm on the glue surface, continue to press the column according to the flow rate above until the glue surface is clear and stable, mark the position of the glue surface.
- Stop the pump, open the valve/plug on the plunger head, close the valve/plug on the bottom of the column, slightly relax the plunger head sealing ring, press the plunger head down to about 0.3cm below the marked position, tighten the plunger head sealing ring, close the valve/plug on the plunger head, and the loading of the column is completed.

2.2 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 2: 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

2.3 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

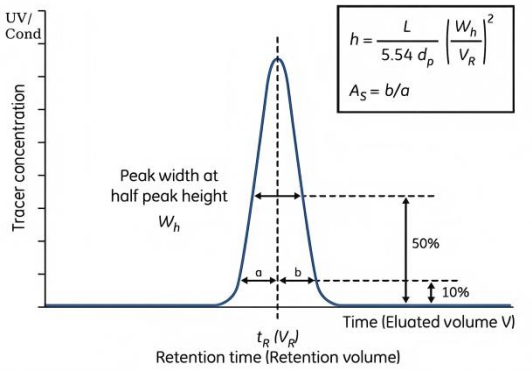
N=theoretical plate number

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



2.4 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.

2.5 Chromatographic methods

- **Recommended buffer:**
  - Binding buffer: 0.1M Tris, 10mM EDTA, 2.1M $(NH_4)_2SO_4$, pH7.5
  - Elution buffer: 0.1M Tris, 10mM EDTA, 2.0M $(NH_4)_2SO_4$, pH7.5
  - Elution buffer: 0.1M Tris, 10mM EDTA, 1.7M $(NH_4)_2SO_4$, 0.3M NaCl, pH 7.5

  In some cases, 0.6-0.8 M sodium sulfate can be replaced by 0.5 M potassium sulfate.
- **Flow rate:** According to the height of the column generally choose a flow rate of 50~120cm/h, the higher the column height the slower the flow rate.
- **Sample and sample volume:** In order to prevent the sample from clogging the column, the sample needs to be filtered with 0.45μm microporous filter membrane and the pH and conductivity of the sample should be adjusted to be consistent with the equilibrium buffer before the sample is loaded.
- **Equilibration:** Fully equilibrate the chromatography column with binding buffer, usually 3~5 column volumes are needed.
- **Sampling:** Determine the volume of sample according to the loading capacity of the medium and the concentration of superhelical DNA in the sample, usually 80% of the loading capacity.
- **Rinse:** Use the recommended buffer to rinse out the loop-opened plasmid DNA.
- **Elution:** The recommended elution buffer can be used to elute and collect the elution peaks.
- **Regeneration:** Wash the chromatography column with 3CV water, then wash with 3CV 0.5M NaOH.
- **Re-equilibration:** Wash with binding buffer. When the pH and conductivity are essentially the same as that of the equilibration buffer, the column is ready for a second sample, and so on.

2.6Application Case

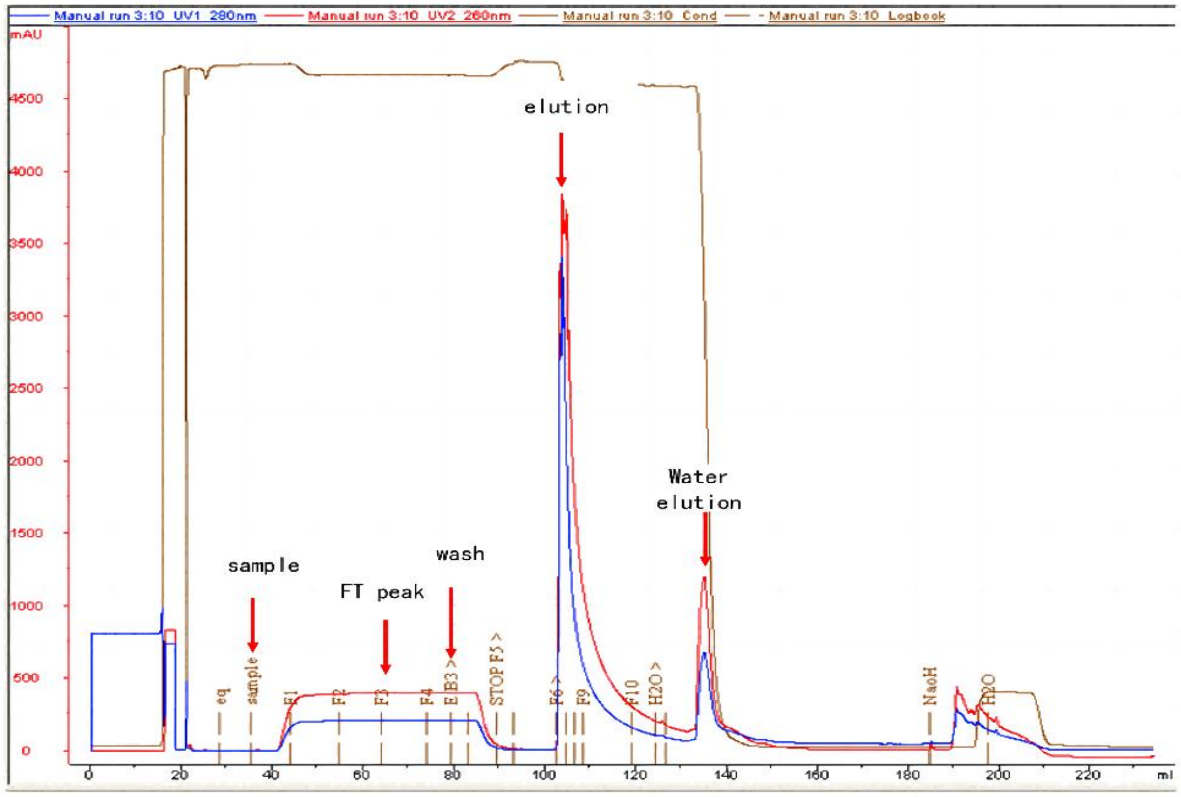
Example: Thiophilic affinity chromatography purification (removal of ring-opened ocDNA using TH-Plasmid Fine)

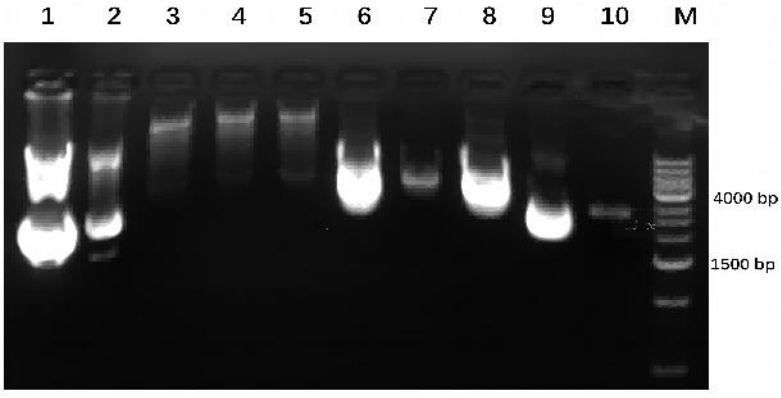
Chromatography column: TK-Col Plasmid Fine-5mL

Equilibrium solution: 2.0M $(NH_4)_2SO_4$, 100mM Tris-HCl, 10mM EDTA, pH=7.5

Elution solution: 0.3M NaCl, 1.7M $(NH_4)_2SO_4$,100mM Tris-HCl, 10mM EDTA, pH=7.5

Sample: peak collection of plasmid DNA captured by gel filtration using TA-6FF in the previous step.





Order of spotting samples:
1: Plasmid DNA samples;
2: TA-6FF Collection Fluid;
3: TH-Plasmid Fine flow-through 1;
4: TH-Plasmid Fine flow-through 2;
5: TH-Plasmid Fine flow-through 3;
6: TH-Plasmid Fine elution peak 1;
7: TH-Plasmid Fine elution peak 2;
8: TH-Q Fine Sampling (affinity eluent);
9: TH-Q Fine Elution Peak Collection Solution 1;
10: TH-Q Fine Elution Peak Collection Solution 2;
M: Marker;
Note: Ammonium sulfate is present in the TH-Plasmid Fine collection solution, so the electrophoretic bands will be shifted.

Affinity chromatography result analysis: The plasmid DNA peak collection of TA-6FF was subjected to TH-Plasmid Fine Sulfophilic Affinity Chromatography, and the yield and purity could reach more than 90%.

3. 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 protein removal: 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.
- Removal of strongly hydrophobic substances or lipids: 2~4 column volumes of 20mM PB+30% isopropanol, pH7.5 buffer were used to clean the column, and purified water was used before and after rinsing.

4. 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.

5. Destruction and recycling

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

6. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TH-Plasmid Fine	Y5071	25mL
	Y5072	100mL
	Y5073	500mL
	Y5074	1L
	Y5075	5L
	Y5076	10L
	Y5077	20L