TH-MC Fine

High Resolution Highly Rigid Multi-Mode Weak Cation Exchange Resin

Product Manual

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Content

1. Product Introduction

Ion exchange chromatography (IEX) is a class of very effective biomolecule separation and purification methods, the method mainly relies on the interaction between positive and negative charges, the use of different biomolecules in specific conditions with the nature of the charge and the number of differences in order to carry out the separation, which has a high loading capacity, good resolution, controllable conditions, easy to amplify features It has been widely used in medicine, chemical industry, metallurgy, food and other fields. However, when doing ion exchange chromatography, the salt content of the sample should not be too high, and the composite ion exchange medium has a certain tolerance to salt by introducing benzene ring on the ligand group, which eliminates the desalting step of the high-salt sample and expands the application scope of ion exchange.

Composite ion exchange media is composed of three parts: (1) cross-linked mesh matrix, the matrix has a porous, hydrophilic, chemically stable characteristics, Truking Hard matrix for the high rigidity of the agarose matrix, is through the traditional TA-6FF matrix of chemical modification and modification, with better mechanical properties; (2) fixed in the matrix of the functional group, which is a complex charge group, which is usually composed of two parts, a charged part and a hydrophobic part; (3) an ion with an opposite charge to the functional group (which can be called a balancing ion), which can be reversibly combined with the ionic functional group.

TH-MC Fine is a multimodal weak cation exchange medium formed by coupling complex groups with benzene rings and carboxyl groups on highly rigid agarose microspheres with an average particle size of 40um.

Table 1 TH-MC Fine Technical Parameters

Appearance	White paste, layered on placement
Framework	Highly rigid agarose
Functional groups	Complex weak cationic groups
Average particle size	40μm (36~44μm)
Ion Carrying Capacity	33~48μmol H^+/mL medium
Pressure resistance	0.5 MPa
Operating pH range	3~12
Chemical Stability	Common aqueous phase solution, 1M NaOH, 6M guanidine hydrochloride, 8M urea, 30% isopropanol, 70% ethanol
pH Stability	2~14 (CIP); 3~12 (working)
Temperature tolerance	Use temperature 4~40℃, can't freeze
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol
Recommended Flow Rate	60~300cm/h

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-MC 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.

- Replacement solution: Shake the suspension well and measure the volume calculated by the above method, pour it into the funnel, draw off the liquid and wash it with about 3mL of mounting solution (20% ethanol + 0.4M NaCl)/mL of medium, repeat the washing 3 times, each time when adding the washing solution, you need to use a glass rod or stirring stick to stir in order to wash off the original preservation solution better.
- Gel suspension preparation: Transfer the washed medium from the funnel to a beaker or other suitable container, add the column loading 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 (using a column loader if necessary), taking care not to introduce 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).
- When the column height is 15~20cm, the loading flow rate can be set to 450cm/h. Open the bottom valve/plug of the chromatography column, turn on the flow rate, and press the column using the set flow rate until the gel surface is clear and stable, and mark the position when the gel surface is stable.
- Remove the column loader (if any), install the upper column head, lower the column head to about 0.5 cm above the gel surface, continue to press the column at the above flow rate until the gel surface is clear and stable, and then mark the column height at the time of gel surface stabilization.
- Stop the pump, open the valve/plug on the column head, close the valve/plug at the bottom of the column, slightly relax the sealing ring of the column head, press down the column head to about 0.3cm below the marking position, tighten the sealing ring of the column head, close the valve/plug at the column head, and the installation 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

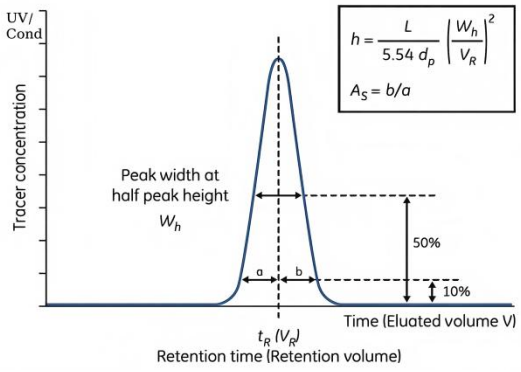
N=theoretical plate number

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

- **Buffer selection:** buffer salts whose buffer groups do not interact with the medium should be selected; low salt and low pH (usually 1 pH unit below the isoelectric point of the target) buffers are preferred for equilibrium buffers to favor the binding of the target, and the stability of the target in the buffer needs to be considered.The elution buffer is usually a buffer with a high concentration of salt (e.g. 1M NaCl) added to the equilibration buffer or a high pH elution.
- **Flow rate:** According to the height of the column generally choose the flow rate of 60~300cm/h, the larger the column height the slower the flow rate.
- **Sample Preparation:** To prevent the sample from clogging the column, the sample needs to be filtered through a 0.45 μm microporous membrane and the pH and conductivity of the sample adjusted to match that of the equilibration buffer prior to sampling (the pH and conductivity of the sample can be adjusted by

dilution, ultrafiltration, and replacement of the buffer with TD-G25).

- **Equilibration:** The pH and conductivity of the buffer used to flush the chromatography column to the outlet with equilibration buffer is essentially the same as that of the equilibration buffer, and usually requires 3 to 5 times the column bed volume.
- **Sampling:** The volume of the sample is determined according to the content of the substance in the sample and the binding load of TH-MC Fine.
- **Rinse:** Rinse the column with equilibration buffer until the UV absorption is close to baseline.
- **Elution:** 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 and collect different fractions to detect where the target is located.
- **Regeneration:** Flush the chromatography column with a salt containing a high concentration (e.g. 2M NaCl).
- **Re-equilibration:** Rinse with the equilibration buffer, and when the pH and conductivity are essentially the same as the equilibration buffer, a second sample can be taken, and so on.

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 prevents the accumulation of contaminants and maintains a stable working condition. Determine the frequency of in-situ cleaning according to the degree of contamination of the media (if contamination is severe, it is recommended that in-situ cleaning be performed after each use to ensure reproducibility of results).

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

- Removal of tightly bound proteins: Wash with 2~3 column volumes of 2M NaCl.
- For the removal of strongly hydrophobic proteins and precipitated proteins: first wash with 2-3 column volumes of 1M NaOH, then immediately rinse with 5-10 column volumes of pure water.
- Removal of lipoproteins and lipids: Wash with 5-10 column volumes of 70% ethanol or 30% isopropanol, followed by a rinse with 5-10 column volumes of pure water.
- Cleaning can also be carried out by combining the above two cleaning conditions, i.e., cleaning with a 30% isopropanol solution containing 1M NaOH.

Note: 70% ethanol or 30% isopropanol should be degassed before use; the flow rate can be selected from 30-60cm/h during bit cleaning; reverse cleaning can be used when the blockage is serious.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution containing 0.2M sodium acetate does not have sterilizing and de-pyrogenic effects, it is recommended that TH-MC Fine be treated with 1M NaOH for 0.5~1h before and during use to reduce the risk of microbial contamination.

TH-MC Fine is sold in 20% ethanol or 2% benzyl alcohol containing 0.2M sodium acetate as preservation solution. After use, TH-MC Fine should be stored in 20% ethanol containing 0.2M sodium acetate in a closed container at 2-30°C. It is recommended that the preservation solution be replaced with fresh preservation solution every 3 months to prevent ethanol volatilization and microbial growth.

5. Destruction and recycling

Since TH-MC Fine is difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TH-MC Fine	Y2152	25mL
	Y2153	100mL
	Y2154	500mL
	Y2155	1L
	Y2156	5L
	Y2157	10L
	Y2158	20L