TH-MA

High-Rigidity Multimodal Strong Anion 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) crosslinked mesh matrix, the matrix has a porous, hydrophilic, chemically stable, Truking Hard matrix is a high rigidity agarose matrix, which is chemically reconfigured and modified from the traditional TA-6FF matrix, with better mechanical properties; (2) fixed on the matrix of functional groups, which is the compositecharge 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-MA is a multimodal strong anion exchange resin formed by coupling two groups, phenyl glycidyl ether and 2,3-epoxypropyltrimethylammonium chloride, respectively, to highly rigid agarose microspheres through two cross-links.

Table 1 TH-MA technical parameters

Appearance	White paste, layered on placement
Framework	Highly rigid agarose
Functional groups	Complex strong anionic groups
Average particle size	75μm
Ion Carrying Capacity	90~120μmol Cl^-/mL filling medium
Pressure resistance	0.5 MPa
Operating pH range	3~12
Chemical stability	Common aqueous solutions: 1M $NaOH^+$, 1M HAc^+, 10mM NaOH, 6M guanidine hydrochloride, 8M urea, 70% ethanol, 30% isopropanol, 20% ethanol, 2% benzyl alcohol Avoid contact with oxidizing agents and anionic detergents.
pH Stability	2~14 (CIP); 3~12 (working)
Temperature tolerance	Use temperature 2~40℃, can't freeze

Storage ++	2~30℃, 20% ethanol or 2% benzyl alcohol
Recommended Flow Rate	90-500cm/h

+1M NaOH and 1M HAc for cleaning only.

++2% Benzyl Alcohol for foreign shipments only or as specified by the customer.

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-MA needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.12 (i.e., compression ratio of approximately 1.12)

  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 column solution (20% ethanol + 0.4M NaCl)/mL of media, repeat the washing 3 times, each time when adding the washing solution, it is necessary 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 cleaned medium from the funnel to a beaker or other suitable container, add the column filling solution to a concentration of 50~75% of the gel suspension, stir well and set aside.
- Take cleaned TK-EC chromatography columns (TK-EC series chromatography columns with different specifications from 1cm to 45cm in diameter can satisfy chromatography applications of different scales and sizes), drain the membrane air bubbles at the bottom of the column and keep about 1cm high water at the bottom of the column, tighten the lower plug, and adjust the column so that it is perpendicular to the ground.
- Slowly pour the stirred gel suspension into the chromatography column one at a time (using a column loader if necessary), taking care not to bring in air bubbles, and stirring again with a stirring rod after pouring.
- 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 (TH-MA can be set to 500cm/h for a column loading height of 15~20cm), open the bottom valve/plug of the chromatography column, turn on the flow rate, and use the set flow rate to press until the adhesive surface is clear and stable, and mark the position of the adhesive surface when it is stable.
- Remove the column loader (if there is one), lower the column head to about 0.5 cm above the gel surface, continue to press the column at the flow rate above until the gel surface is clear and stable, and then mark the column height at the time the gel surface is stable.

- Stop the pump, open the valve/plug on the plunger head, close the valve/plug on the bottom of the plunger, slightly relax the sealing ring of the plunger head, press down the plunger head to about 0.3cm below the marking position, tighten the sealing ring of the plunger head, close the valve/plug of the plunger head, and the loading of the plunger 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

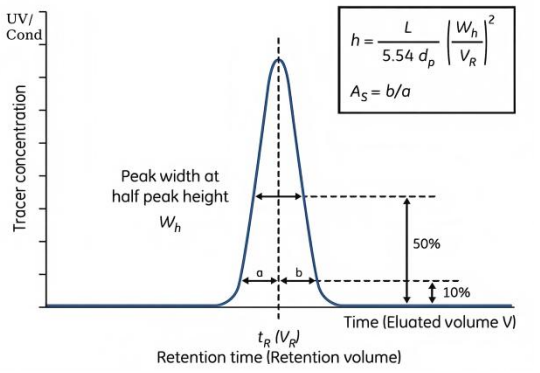
N=theoretical plate number

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. The pH of the equilibrium buffer is usually 1 pH unit above the isoelectric point of the target, and the buffer is designed to favor the binding of the target, while the stability of the target in the buffer needs to be considered. Elution buffer needs to be determined according to the actual situation, if the main principle of binding is ion exchange, usually add a high concentration of salt in the equilibrium buffer (e.g., 1M NaCl) or

low pH to elute, if hydrophobicity plays a certain role in the binding, you need to choose a low salt and low pH buffer as the elution buffer.

- **Flow rate:** According to the height of the column generally choose 90~500cm/h flow rate, the higher 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 (pH and conductivity 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-MA, and the sampling is carried out.
- **Wash:** Wash the column with equilibration buffer until the UV absorption is close to the 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:** wash the column with a buffer containing a high concentration of salt (e.g. 2M NaCl)
- **Re-equilibration:** Wash with the equilibration buffer, and when the pH and conductivity are essentially the same as the equilibration buffer, the 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 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).

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

- Removal of more tightly bound proteins: wash with 2~3 column volumes of 2M NaCl.
- Removal of strongly hydrophobic proteins, precipitated proteins: first wash with 2-3 column volumes of 1M NaOH, then immediately wash with 5-10 column volumes of purified water.
- Removal of lipoproteins and lipids: Wash with 5-10 column volumes of 70% ethanol or 30% isopropanol followed by 5-10 column volumes of purified water.
- Can also be cleaned by combining the two cleaning conditions described above, i.e., 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 does not have sterilizing and de-pyrogenic effects, it is recommended that TH-MA can be treated with 1M NaOH for 0.5~1h to reduce the risk of microbial contamination before and during use.

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

5. Destruction and recycling

Since TH-MA 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-MA	Y2124	25mL
	Y2125	100mL
	Y2126	500mL
	Y2127	1L
	Y2128	5L
	Y2129	10L
	Y2130	20L