TH-Phenyl(HS)

Highly Rigid Agarose Hydrophobic Chromatography Resin

Product Manual

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Content

1. Product Introduction

Hydrophobic interaction chromatography (HIC) is a chromatographic method that utilizes the interaction between hydrophobic groups carried by biomolecules and hydrophobic ligands on the stationary phase to separate substances. Salt ions can disrupt the hydration membrane on the surface of biomolecules and promote the binding between hydrophobic groups and ligands.

TH-Phenyl (HS) is a medium with high rigidity agarose as a base frame coupled with highly substituted strongly hydrophobic phenyl groups, which is suitable for large-scale separation and purification of biomolecules due to its low backpressure and fast flow rate.

Table 1 TH-Phenyl (HS) Technical Parameters

Appearance	White paste, layered on placement
Framework	Highly Rigid Agarose
Functional Groups	Phenyl
Ligand density	~27μmol ligand/mL Medium
Particle size range	40~120μm
Dynamic loading	~27mg BSA/mL media
Pressure resistance	0.5MPa
Pressure flow rate	≥1200cm/h (0.5MPa TK-EC 100/500 h=20cm Temperature 20°C)
Chemical stability	Common aqueous phase solutions: 1M NaOH, 1M HAc, 6M guanidine hydrochloride, 30% isopropanol, 70% ethanol
pH stability	2~14 (CIP, short term), 3-13 (working)
Temperature Tolerance	Use temperature 2~40℃, can not be frozen, resistant to 121℃, 20min sterilization
Storage	2-30℃, 20% ethanol or 2% benzyl alcohol
Recommended Flow Rate Range	150-350cm/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-Phenyl(HS) 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 Wash: 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 filling solution (20% ethanol)/mL of media, repeat the washing 3 times, each time when adding the wash solution, it is necessary to stir it with a glass rod or stirring stick in order to wash off the original preservation solution better.
- Gel suspension preparation: In order to get a better column loading effect, the concentration of the gel suspension should be between 50% and 75%, i.e., the volume of the sinking gel is one-half to three-quarters of the total volume, 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, and stirring again with a plastic 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 to 200cm/h, open the lower head of the column, and start the peristaltic pump or chromatography system according to the flow rate set above. Set the alarm pressure less than 0.3MPa, if the pressure exceeds 0.3MPa during column loading, the flow rate needs to be reduced appropriately.
- Remove the column loader (if there is one), lower the column head to a position about 0.5 cm above the gel surface, and continue to press the gel once at a flow rate of 600 cm/h, marking the position of the gel surface.
- Stop the pump, loosen the plunger seal slightly, make sure the valve on the plunger head is closed and the valve at the bottom of the column is open, lower the glue surface to 5mm below the marked position, screw the plunger seal tightly, close the valve at the bottom, and the column loading 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

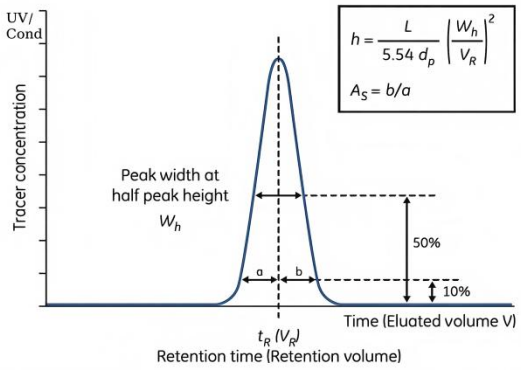
N=theoretical plate number

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

Note: Temperature has a strong influence on hydrophobic chromatography. Keeping the temperature of the environment, the buffer and the samples the same during the experiment is the only way to ensure the reproducibility of hydrophobic chromatography.

- **Buffer selection:** the binding buffer is usually a phosphate buffer containing a high concentration of salts, such as 20 mM PB, 1.5 M $(NH_4)_2SO_4$, pH 7.0. The elution buffer is usually a phosphate buffer without other salts, such as 50 mM PB, pH 7.0, which needs to be adjusted according to the results of the subsequent experiments (whether or not there is a precipitation of the target species, the binding of the target species is strong or weak, the recovery, the degree of separation, etc.).The concentration and type of salt in the binding buffer need to be adjusted according to the results of subsequent experiments. For substances that are more difficult to elute, pure water can be used, or a low concentration of ethanol can be added to pure water as the

eluent.

- **Sample and sample volume:** The pH and conductivity of the sample need to be adjusted to match that of the binding buffer, and in order to prevent the sample from clogging the column, the sample needs to be filtered through a 0.45 μm microporous membrane prior to sampling, and the sample volume is determined according to the content of the substances in the sample and the binding loading capacity of TH-Phenyl(HS).
- **Equilibration:** Wash the column with equilibration buffer until the pH and conductivity of the buffer at the outlet are basically the same as that of the equilibration buffer, usually 3 to 5 column bed volumes are needed.
- **Sampling:** Prepare the sample for sampling according to the set conditions.
- **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 or decrease the strength of salt ions in the eluent, or add detergent to the buffer to elute substances with different binding strengths from the column, collect different components, and detect the location of the target.
- **Regeneration:** Rinse the chromatography column with purified water or 30% isopropanol (70% ethanol).
- **Re-equilibration:** After rinsing with equilibration buffer the column is ready for a second sample and so on.Rinse with equilibration buffer and when pH and conductivity are in general agreement with the equilibration buffer, the column is ready for a second sample, 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 more tightly bound proteins: wash with 2~3 column volumes of purified water.
- 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.

The plastic housing of the laboratory type TK-EC minicolumns is not resistant to organic solvents such as 70% ethanol, so care should be taken not to spill organic solvents on the housing during use.

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-Phenyl(HS) can be treated with 1M NaOH for 0.5~1h to reduce the risk of microbial contamination prior to and during use, or it can be sterilized by 121℃, 20min autoclaving.

TH-Phenyl(HS) is sold with 20% ethanol or 2% benzyl alcohol as preservation solution. After use, TH-Phenyl(HS) should be stored in 20% ethanol at 2~30°C in airtight condition. In order to prevent volatilization of ethanol and microbial growth, it is recommended to replace the preservation solution with fresh one every 3 months.

5. Destruction and recycling

Since TH-Phenyl(HS) 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-Phenyl (HS)	Y3057	25ml
	Y3058	100ml
	Y3059	500ml
	Y3060	1L
	Y3061	5L
	Y3062	10L
	Y3063	20L