TA-Heparin HP

Affinity Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA-Heparin HP is a race-specific affinity chromatography medium made by covalently coupling heparin to high cross-linking agarose. It is widely used for the separation and purification of various biomolecules, especially enzymes, including antithrombin III, thrombin-like enzymes, human coagulation factors IX, XI and VIII, lipoprotein lipases, collagenases and DNA polymerases, and it can also be used for the separation and purification of human interleukin, human prostate growth factor, recombinant human vascular endothelial growth factor, chondrocyte growth factor, basic fibroblast growth factor, recombinant human acidic fibroblast growth factor, recombinant hepatocyte growth factor, recombinant murine cofactor II, recombinant human platelet factor IV, recombinant human endothelial inhibitor and recombinant human keratinocyte growth factor, etc. The medium is characterized by physical and chemical stability, non-shedding of ligands, long service life, wide range of applications and easy scale-up.

Table 1 TA-Heparin HP technical parameters

Appearance	White slurry, layered on placement
Base Frame	Highly cross-linked 6% agarose
Average particle size	34μm (24~44μm)
Functional groups	Heparin
Ligand density	~10mg Heparin ligand/mL medium
Chemical Stability	Stable in all commonly used water-soluble buffers: 8M urea, 6M guanidine hydrochloride, 70% ethanol, 50mM sodium acetate (pH4), 10% glycerol, 0.1M NaOH (20°C for one week)
pH Stability	5~10
Temperature resistance	Use temperature 2~30℃, can not be frozen, can be 121℃ autoclaved for 30min (high pressure with 20mM NaH2PO4, pH7.5 cycle)
Pressure resistance	0.3MPa
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol, 50mM sodium acetate
Recommended Flow Rate	60~300cm/h

+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 TA-Heparin HP needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.15 (i.e., compression ratio of approximately 1.15)

  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.

- Wash the medium: Shake the medium 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 the medium, repeat the washing 3 times, each time you add 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 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 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 10~20cm, set the loading flow rate to 60cm/h, open the bottom valve/plug of the chromatography column, start the pump and press the column using the set flow rate until the gel surface is clear and stable, mark the position of the gel surface when it is stable.
- Remove the column loader (if present), lower the column head to a position approximately 0.5 cm above the gel surface, set the flow rate to 300 cm/h and continue to press the column until the gel surface is clear and stable, mark the position of the gel surface.
- 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 the column head down to about 0.3cm below the marked position, tighten the sealing ring of the column head, close the valve/plug of the column head, 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

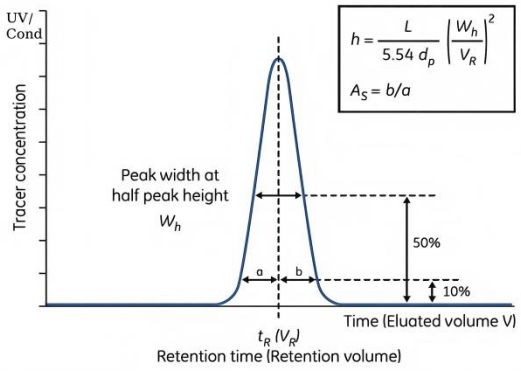
N=theoretical plate number

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

- **Buffer selection:**
  - binding buffer: 20-50 mM PB or Tris, pH 7.4-8.0, 0.15 M NaCl can be added to inhibit non-specific adsorption.
  - elution buffer: 20-50 mM PB or Tris, 1-2 M NaCl, pH 7.4-8.0, NaCl concentration needs to be adjusted appropriately according to the binding strength of the target protein.
- **Flow rate:** According to the height of the column generally choose 60~300cm/h flow rate, the higher the column height the slower the flow rate.
- **Sample and sample volume:** To prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous filter membrane and the pH and conductivity of the sample should be adjusted to be consistent with the equilibrium buffer before sampling, and the volume of the sample should

be determined according to the content of the substances in the sample and the binding loading of TA-Heparin FF.

- **Wash:** Flush the chromatography column with equilibration buffer until the UV absorption at the outlet 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 chromatographic column.
- **Regeneration:** Wash the chromatography column with a buffer containing a high concentration of salt (e.g. 2M NaCl).
- **Re-equilibration:** Re-equilibration: Wash with 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.Customers can determine the frequency of in-situ cleaning according to the degree of media contamination during use (if contamination is severe, it is recommended that in-situ cleaning be performed after each use to ensure reproducible results).

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

- **Removal of ion-bonded proteins:** wash with 3-5 column volumes of 2M NaCl followed by 3-5 column volumes of pure water.
- **Removal of precipitated or denatured proteins:** Wash with 2 column volumes of 0.1M NaOH, followed by 5-10 column volumes of pure water, or 6M guanidine hydrochloride or 8M urea.
- **Removal of hydrophobically bound proteins from:** 0.1-0.5% nonionic detergent wash followed by 3-5 column volumes of pure water.

Note: The flow rate can be selected from 30-60cm/h during bit cleaning, and reverse cleaning is recommended.

For immediate use after cleaning, rinse 3-5 column volumes with equilibration buffer and use, or if not to be used for a short period of time, wash 3-5 column volumes with 20% ethanol solution and store them.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution containing 50mM sodium acetate is not bactericidal or pyrogenic, it is recommended that TA-Heparin HP can be treated with 70% ethanol for more than 12h before and during use to reduce the risk of microbial contamination.

TA-Heparin HP is sold in 20% ethanol or 2% benzyl alcohol containing 50 mM sodium acetate as a preservation solution. After use, TA-Heparin HP should be stored in 20% ethanol containing 50 mM sodium acetate, airtight at 2-30° C. To prevent ethanol evaporation and microbial growth, it is recommended that the preservative be replaced with a fresh solution every 3 months.

5. Destruction and recycling

Since TA-Heparin HP 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
TA-Heparin HP	Y5127	25mL
	Y5128	100mL
	Y5129	500mL
	Y5130	1L
	Y5131	5L
	Y5132	10L
	Y5133	20L