TH-Protein L

Affinity Chromatography Resin

Product Manual

楚天微球生物技术(长沙)有限公司
TRUKING MICRO-SPHERE BIOTECHNOLOGY (CHANGSHA) CO., LTD

Content

1. Product Introduction

TH-Protein L is a novel affinity medium synthesized by epoxidation of a certain base-resistant Protein L with a highly rigid agarose substrate, with an optimized pore size that facilitates the increase of antibody binding load. The medium is suitable for binding the variable region of the kappa light chain of the antibody, and is suitable for capturing a wide range of antibody fragments such as Fabs, single-chain variable fragments (scFv) and antibody domains (dAbs).

The ligand of TH-Protein L is a genetically engineered recombinant protein L fragment with a certain degree of alkali resistance, and the ligand fermentation and subsequent purification processes are performed without any animal-derived materials. The medium can be cleaned in situ with 15 mM NaOH, avoiding the use of expensive and corrosive in situ cleaning reagents, which can effectively save costs. Meanwhile, TH-Protein L has a higher relative loading capacity, so that a smaller column volume can be selected to reduce the cost of production during the process of scaling up.

The medium has the following characteristics:

- Highly rigid agarose base frame for fast flow rates for increased productivity.
- Highly specific kappa light chains for efficient capture of a broad selection of antibodies and antibody fragments.
- High loading capacity reduces process time and media usage, resulting in lower production costs and increased productivity.

Table 1: TH-Protein L technical parameters

Appearance	White paste, layered on placement
Base Frame	Highly rigid agarose
Average particle size	~80μm
Functional groups	Recombinant Protein L (from E.coli)
Crosslinking mode	Epoxy Chemistry
Dynamic binding load	~40mg human IgG/mL media (contact time 6min)
Chemical stability	Common aqueous phase solutions: 10mM HCl, 0.1M citric acid (pH3.0), 6M urea, 6M guanidine hydrochloride, 30% isopropanol, 20% ethanol
Pressure resistance	0.5MPa
Maximum flow rate	≥300cm/h, TK-EC300, H=20cm
pH stability	2-10 (working); 15mM NaOH (CIP)
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-Protein L 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: Pour the gel suspension into a Brinell funnel, draw off the liquid, and wash with about 3 times the volume of the loading solution (20% ethanol containing 0.4M NaCl), (when the volume is relatively large or the conditions are not available can also be used to wait for the gel to stratify the upper layer of the solution, and then add appropriate amount of loading solution stirring and wait for the stratification of the solution to be withdrawn, and then repeat the method of replacing the media solution for 3 times).
- Preparation of Column Mounting Gel Suspension: 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 sedimentation gel should be 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.
- 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 pump flow rate according to the linear flow rate of 200cm/h, open the valve at the bottom, start the pump to press the glue until the glue surface is stable (pay attention to the setting of the alarm pressure less than 0.3MPa or pay attention to the pressure in the column at any time to prevent overpressure in the column).
- After the glue surface is stabilized, keep it for more than 10min, mark the position of the glue surface, and then stop the pump.
- Remove the column loader (if there is one), lower the column head to a position about 0.5cm above the glue surface, and continue to press the glue once at a flow rate of 360cm/h, marking the position of the glue surface.
- Stop the pump, slightly loosen the column head sealing ring, make sure the valve on the column head is closed and the valve at the bottom of the column is open, press down the glue surface to 5mm below the marked position, screw the column head sealing ring 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

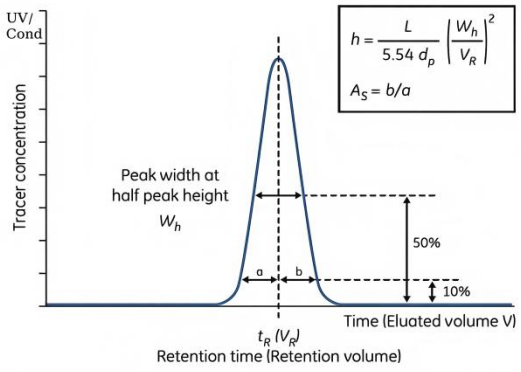
N=theoretical plate number

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

- **Buffer:** A neutral buffer is usually used as the binding buffer (e.g., 20 mM PB, 0.15 M NaCl, pH 7.2), and a low pH buffer is used as the elution buffer (e.g., 0.1 M sodium citrate pH 2.0-3.5), and when optimizing the elution conditions, it is necessary to map out the highest pH for effective desorption to avoid denaturation of unstable antibodies caused at too low a pH.
- **Flow rate:** According to the height of the column usually choose a linear flow rate of 60~300cm/h, the higher the column height the slower the flow rate.
- **Equilibration:** Equilibrate the column with equilibration buffer, usually 4~6 column volumes are needed.
- **Sample Preparation:** To prevent the sample from clogging the column, the sample needs to be filtered with 0.45μm microporous membrane and the pH and conductivity of the sample should be adjusted to be

consistent with that of the equilibration buffer before loading the sample.

- **Sampling:** The volume of the sample is determined by the amount of material in the sample and the binding capacity of TH-Protein L. The volume of the sample is determined by the binding capacity of the TH-Protein L.
- **Wash:** After the sample is loaded, wash the sample with binding buffer for 1~5CV to bring the UV absorption down to a suitable value. If necessary, a high salt or slightly lower pH wash can be added to wash down as many non-specifically adsorbed impurities as possible.
- **Elution:** A 10CV linear gradient from binding solution to elution buffer (e.g. 0.1M citric acid-sodium citrate buffer, pH 3.0) can be used to determine the optimal pH for elution based on the location of the antibody peak. If the antibody is unstable under acidic conditions, the eluate can be neutralized with a neutralizing solution (e.g., 1.0M Tris).
- **Regeneration:** Wash the chromatography column with 5~10CV elution buffer.

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).

- First wash with binding buffer for 2~3CV;
- Then wash 2~3CV with 15mM NaOH for 10~15min contact time;
- Immediately wash with binding buffer (pH 7~8) for at least 5CV.

Note: If antibodies bound to the media do not elute completely, they should be regenerated prior to CIP. It is recommended to equilibrate the chromatography column with a neutral pH solution prior to CIP with NaOH, to avoid the possibility of elevated column temperatures that may result from direct contact between a low pH buffer and a high pH NaOH solution.

4. Sterilization and storage

TH-Protein L is sold in 20% ethanol or 2% benzyl alcohol as a preservation solution. After use, TH-Protein L should be stored in 20% ethanol at 2-8°C in a closed container. To prevent ethanol evaporation 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-Protein L is difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 4 Article number and packaging

Product	Item No.	Norm
TH-Protein L	Y5214	25mL
	Y5215	100mL
	Y5216	500mL
	Y5217	1L
	Y5218	5L
	Y5219	10L
	Y5220	20L