TK-Col Hard Protein A
TK-Col 10/10 Hard Protein A
TK-Col 16/10 &16/20Hard Protein A
TK-Col 26/10 Hard Protein A

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TH-Protein A is a new type of affinity chromatography medium made by coupling alkali-resistant Protein A with a highly rigid agarose substrate through epoxy activation. Compared with traditional antibody affinity media, it has a more stable ligand, faster flow rate, and lower backpressure, and is suitable for the capture of monoclonal antibodies or Fc fusion proteins from high-volume cell cultures, and the capture of polyclonal antibodies from peritoneal fluid or blood plasma. It is also suitable for capturing polyclonal antibodies from ascites or plasma.

The ligand of TH-Protein A is a recombinant protein A fragment obtained by E. coli fermentation. In the upstream construction, the base-resistant amino acids are replaced by the non-base-resistant amino acids, and the protease-sensitive amino acids are removed, which makes the ligand with good stability, and the fermentation of the ligand and the subsequent purification process use no raw materials of animal origin. The medium can be cleaned in-situ with 0.1-0.5M NaOH, avoiding the use of expensive and corrosive cleaning reagents, which can effectively save the cost and is easy to be used in industrialized large-scale production.

TK-Col Hard Protein A preloaded columns are ready-to-use affinity chromatography columns filled with TH-Protein A media in TK-EC 1 ml ,TK-EC 4.9 ml ,TK-EC 5 ml ,TK-EC 20 ml chromatographic vacutainer columns. TK-Col 10/10 Hard Protein A preloaded columns are ready-to-use affinity chromatography columns filled with TH-Protein A media in TK-EC 10/20 columns; TK-Col 16/10 Hard Protein A preloaded columns are ready-to-use affinity chromatography columns filled with TH-Protein A media in TK-EC 16/20 columns; TK-Col 16/20 Hard Protein A preloaded columns are ready-to-use affinity chromatography columns filled with TH-Protein A media in TK-EC 20 ml columns. - TK-Col 16/20 Hard Protein A preloaded columns are ready-to-use affinity columns filled with TH-Protein A media in TK-EC 16/30 columns; TK-Col 26/10 Hard Protein A preloaded columns are ready-to-use affinity columns filled with TH-Protein A media in TK-EC 26/20 columns. This series of columns eliminates the hassle of loading the columns by the customer and the risk of poor column performance. This type of preloaded columns is widely used in laboratory process development, small amount of sample preparation, and is suitable for the separation and purification of biomolecules such as antibodies and Fc fusion proteins. It has the following features:

- Ready-to-use
- Volume stabilization of the column bed
- Good physical and chemical resistance

2. Technical parameters

Table 1: TH-Protein A technical parameters

Appearance	White paste, layered on placement
Base frame	Highly rigid agarose
Average particle size	75μm
Functional group	Recombinant alkali-resistant Protein A (24kD)
Crosslinking mode	Epoxy chemical
Dynamic binding load	>55mg human IgG/mL medium
Chemical stability	TH-Protein A medium is stable in all commonly used buffers; resistant to 10mM HAc, 0.1M sodium citrate, 6M guanidine hydrochloride, 8M urea, 30% isopropanol wash, and can be stored for a long time in 20% ethanol.
Pressure resistance	0.5MPa
Pressure flow rate	300cm/h, <2bar, TK-EC 300/500, H=20cm
pH stability	3-12 (working); 2-13 (CIP, short-term); CIP: 0.1-0.5M NaOH
Storage	2~8°C, 20% ethanol or 2% benzyl alcohol
Recommended Flow Rate	60~300cm/h
Note	The load of the resin hardly changes within 30 days at room temperature, so please feel free to use our samples. However, long-term storage beyond 30 days is recommended at 2~8℃ .

Table 2: Technical parameters for each pre-assembled column (see end page for item number)

Product name	Prepacked resin	Prepacked column volume ml	Inner diameter× Column bed height mm×mm	Recommended flow rate + ml/min	Storage	Pressure resistance	Sieve plate aperture (μm)
TK-Col Hard Protein A	TH- Protein A	1	7×25	<2	2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments).	0.3MPa（3bar）	10
		4.9	8×100	<2			
		5	16×25	<10			
		20	16×100	<10			
TK-Col 10/10 Hard Protein A		7.5-8.2	10×100（±5）	1-4			
TK-Col 16/10 Hard Protein A		19.1-21.1	16×100（±5）	3-10			
TK-Col 16/20 Hard Protein A		38.2-42.2	16×200（±5）	3-10			
TK-Col 26/10 Hard Protein A		50.4-55.7	26×100（±5）	8-26			

3. Methods of use

- *TK-Col 16&26 series chromatography columns are made of glass and should be handled gently to prevent breaking or affecting the column efficiency.*
- *To avoid clogging the column, all samples and buffers need to be filtered through 0.45um membrane.*
- *In order to get a good separation effect, avoid too much temperature difference between the buffer and the column.*
- *Keep the column out of direct sunlight.*
- *Chromatography columns can be used in a chromatography cooler, but the flow rate needs to be reduced appropriately.*

3.1 Connecting the column to the chromatography system

- Open the package and take out the column
- Check whether the column is intact, and whether the column has been dried out during transportation, if any of the above situations occurs, please contact Chutian Microsphere sales representative in time.
- Fix the column next to the chromatography system and pay attention to the flow direction of the column.
- Start the chromatography system, make sure the air bubbles in the chromatography system are drained, and set the alarm pressure of chromatography system to 0.3MPa, then adjust and keep the flow rate running at 0.2ml/min.
- After the chromatography system is purged of air bubbles, open the upper and lower plugs of the chromatography column and connect the chromatography column under low flow rate operation.

3.2 Pretreatment of chromatography columns

- Rinse, the chromatography column is stored in 20% ethanol or 2% benzyl alcohol (for international transportation) during transportation, first rinse off the storage solution with 2 column volumes of distilled water.
- Sterilization, for sample safety, it is recommended to rinse 2 column volumes with 0.5M NaOH before rinsing 2 column volumes with distilled water for the first use.

3.3 Equilibration of Chromatographic Columns

- Buffer selection: a neutral buffer is usually used as the binding buffer (e.g., 50 mM PB, 0.15 M NaCl, pH 7.0), and a low pH buffer is used as the eluent (e.g., 0.1 M citric acid, pH 3.5). since the ability of Protein A to bind to IgG is dependent on the source of the antibody and the subtype (Table 3), high salt and high pH promote antibody and mediator binding and reduce nonspecific binding, and increasing pH neutralizes histidine residues relative to the base-resistant Protein A and IgG binding sites. The electrostatic repulsive effect of these residues impedes the affinity response. Increase salt concentration to reduce electrostatic repulsion and enhance hydrophobicity. Optimization of binding and washout conditions can be performed for different antibodies by changing the type and concentration of salt in the buffer as well as the pH.
- Flush the chromatography column with equilibrium buffer using the recommended flow rate. The pH

and conductance of the buffer to be exported are consistent with the buffer before entering the column, which means the column is well equilibrated, and generally requires 2 to 5 column volumes.

3.4 Flow rate

- Depending on the type of chromatography column, flow rates within the recommended flow rate range are generally selected, with slower flow rates for higher column heights.

3.5 Sampling

- Sample and sample volume: The pH and conductivity of the sample need to be adjusted to be consistent with the binding buffer, and in order to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous filtration membrane before sampling, and the volume of the sample is determined according to the impurity content in the sample and the binding loading of the medium.

3.6 Rinse

- Rinse with equilibration buffer until the UV absorption value drops to the appropriate value.

3.7 Eluent

- When optimizing the elution conditions, it is necessary to map the highest pH for effective desorption to avoid denaturation of unstable antibodies at too low a pH, but too high an elution pH may result in a loss of yield.
- It is usually eluted using a low pH buffer, and the optimization of the elution conditions can be determined using a linear gradient of pH. A 10 column volume linear gradient from equilibrium to elution buffer (e.g., 1 M citric acid, pH 3.0) is usually used to determine the optimal pH for the elution based on the location of the peaks. If the antibody is unstable under acidic conditions, the elution can be neutralized with an appropriate volume of neutralizing solution (e.g., 1.0 M Tris-HCl, pH 9.0).

Table 3 Comparison of affinity of Protein A for specific subtypes of monoclonal antibodies

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG_1	Very high	6.0-7.0	3.5-4.5
IgG_2	Very high	6.0-7.0	3.5-4.5
IgG_3	Low-No	8.0-9.0	≤7.0
IgG_4	Low-High	7.0-8.0	3.0-6.0
Mice			
IgG_1	Low	8.0-9.0	4.5-6.0
IgG_{2a}	Medium	7.0-8.0	3.5-5.5
IgG_{2b}	High	≥7.0	3.0-4.0
IgG_3	Low-High	≥7.0	3.5-5.5

3.8 Regeneration and rebalancing

- Regeneration: Rinse the column with purified water or 30% isopropanol (70% ethanol).
- Re-equilibration: rinsing with equilibration buffer is sufficient for a second sample, and so on.

3.9 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 4: 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

3.10 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

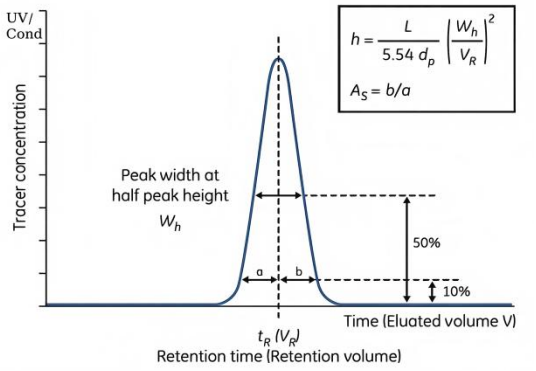
N=theoretical plate number

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



3.11 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.

4. 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).

- The column can be washed for 2 column volumes with 2M NaCl to remove stronger non-specifically bound proteins;
- The column is then washed with 0.1-0.5M NaOH with a contact time of 10-15 min.
- Immediately rinse at least 5 column volumes with binding buffer.

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solutions are not bactericidal or pyrogenic, it is recommended that TH-Protein A media can be treated with 0.1M NaOH for 30min or 0.5M NaOH for 15min prior to and during use to reduce the risk of microbial contamination.

TH-Protein A is sold with 20% ethanol or 2% benzyl alcohol as preservation solution.After use, TH-Protein A should be stored in 20% ethanol at 2~8℃ in airtight condition.To prevent ethanol evaporation and microbial growth, it is recommended that the preservation solution be replaced with a fresh one every 3 months.

6. Destruction and recycling

Since the packing material in the TK-Col Hard Protein A series of pre-packed columns is difficult to degrade in nature, incineration is recommended to protect the environment.

7. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TK-Col Hard Protein A	Y6195	1×1ml
	Y6197	5×1ml
	Y6196	1×5ml
	Y6198	5×5ml
	Y619601	1×4.9ml
	Y619602	1×20ml
TK-Col 10/10 Hard Protein A	Y619901	1pac.
TK-Col 16/10 Hard Protein A	Y6199	1pac.
TK-Col 16/20 Hard Protein A	Y619900	1pac.
TK-Col 26/10 Hard Protein A	Y6200	1pac.