TK-Col FF

Hydrophobic Chromatography Prepacked Columns

Product Manual

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.

TA HIC FF (Fsat Flow) media are made by coupling various hydrophobic chemical groups on highly cross-linked agarose (4% and 6% cross-linking degrees, respectively), which have good physical and chemical stability and can withstand high flow rates to meet the needs of high-throughput industrialized production, and obtain better resolution in the shortest separation time.TA HIC FF mainly includes the following Several types of media:

- TA-Butyl-S
- TA-Phenyl FF(HS)
- TA-Phenyl FF(LS)
- TA-Butyl 4FF
- TA-Octyl 4FF

TK-Col HIC FF series pre-packed columns are ready-to-use pre-packed columns obtained by loading the above chromatography media into the empty columns of TK-EC 1 ml ,TK-EC 4.9 ml ,TK-EC 5 ml ,TK-EC 20 ml, which can save the trouble of loading the columns by themselves and the risk of poor column efficiency, and the perfect combination of the media and the columns makes the chromatography efficient and simple. TK-Col HIC FF series of pre-packed columns are widely used in laboratory process development, media screening and small amount of sample preparation, which are suitable for the separation and purification of biomolecules in the fields of peptides, recombinant proteins and antibodies, etc. TK-Col series of pre-packed columns have the following features:

- Ready-to-use
- Stable column bed volume
- Flexible application
- Good physicochemical tolerance

2. Technical parameters

Table 1: Technical parameters for each type of media

Product name	TA-Butyl-S	TA-Phenyl FF（HS）	TA-Phenyl FF (LS)	TA-Butyl 4FF	TA-Octyl 4FF
Appearance	White paste, layered when placed				
Framework	6% highly cross-linked agarose			4% highly cross-linked agarose	
Functional Groups	-S-$(CH_2)_3$-CH_3（butylthio functional group）	 （Phenyl）	 （Phenyl)	-$(CH_2)_3$-CH_3（butyl）-	-$(CH_2)_7$ -CH_3（octyl）
Ligand density (μmol/ml medium)	≈ 10	≈ 40	≈ 20	≈ 50	≈ 5
Average particle size	90μm				
Pressure Resistance	0.3MPa				
Pressure flow rate	250-400cm/h（100kPa,H=25cm,D=50,25℃）			≥150cm/h100kPa,H=25cm,D=50,25℃）	
pH Stability	2~14 (CIP, short term), 3~13 (working)				
Chemical Stability	Common aqueous phase solutions: 1M NaOH, 1M HAc, 6M guanidine hydrochloride, 30% isopropanol, 70% ethanol, 8M urea				
Temperature resistance	4~40℃, can not be frozen, can withstand 121℃, 20min autoclaving				
volume of cargo	It varies greatly from protein to protein, with a normal range of a few milligrams to over a hundred milligrams per milliliter of media binding				
Recommended Flow Rate Range	150-300cm/h			90-150cm/h	
Storage	2-30℃, 20% ethanol or 2% benzyl alcohol (for international transportation)				

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

Product name	Resin	Column bed volume (ml)	Inner diameter *bed height mm×mm	Recommended flow rate ml/min	Storage	Withstand pressure	Column Material	Sieve plate aperture（μm）
TK-Col Butyl-SFF	TA-Butyl-SFF	1	7×25	0.2-2	2-30° C, 20% ethanol or 2% benzyl alcohol (for international transportation)	0.3MPa（3bar）（43.5psi）	polypropylene	10
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				
TK-Col Phenyl FF (HS)	TA-Phenyl FF (HS)	1	7×25	0.2-2				
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				
TK-Col Phenyl FF (LS)	TA-Phenyl FF (LS)	1	7×25	0.2-2				
		4.9	8×100	0.2-2.5				
		5	16×25	1-10				
		20	16×100	1-10				
TK-Col Butyl 4FF	TA-Butyl 4FF	1	7×25	0.2-2				
		4.9	8×100	0.2-2				
		5	16×25	1-5				
		20	16×100	1-5				
TK-Col Octyl 4FF	TA-Octyl 4FF	1	7×25	0.2-2				
		4.9	8×100	0.2-2				
		5	16×25	1-5				
		20	16×100	1-5				

3. Methods of use

- The chromatography column is made of glass and should be handled gently to prevent it from breaking or affecting the column efficiency.
- To avoid clogging the column, all samples and buffers need to be filtered with 0.45um membrane.
- In order to get a good separation effect, avoid too much temperature change between the buffer and the chromatography column.
- Place the chromatography column in a place without direct sunlight.

3.1 Connecting the column to the chromatography system

- Open the package and take out the chromatography column.
- Check whether the chromatography column is intact, and whether the chromatography column has been dried out by air intake during transportation, if any of the above situations occurs, please contact the sales representative of Truking Micro-sphere in time.
- Fix the chromatography column next to the chromatography system, pay attention to the flow direction of the chromatography column.
- Start the chromatography system, make sure the air bubbles in the chromatography system are drained, and set the alarm pressure of the 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 chromatographic columns

- Rinse, the chromatography column is stored in 20% ethanol or 2% benzyl alcohol (for international shipments) 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 and then 2 column volumes with distilled water for the first use.

3.3 Balancing

- **Buffer selection**: the binding buffer is usually a phosphate buffer containing a high concentration of salts, such as 20mM PB, 1.5M $(NH^4)_2SO_4$, pH7.0. The elution buffer is usually a phosphate buffer without other salts, such as 50mM PB pH7.0, which needs to be adjusted according to the results of the subsequent experiments (whether or not there is a precipitation of the target, the target binding strength, recovery, separation degree, etc.). binding strength, recovery, separation, etc.) the concentration and type of salt in the binding buffer need to be adjusted.Use the recommended flow rate to flush the chromatographic column with equilibrium buffer. The pH and conductivity of the buffer to be exported are consistent with that of the buffer before entering the chromatographic column that means the chromatographic column is well equilibrated, generally 2~5 column volumes are needed.

- **Equilibration:**Use the recommended flow rate to flush the column with the equilibration buffer. The pH and conductivity of the buffer to be exported are consistent with that of the buffer before entering the column, i.e., the column is equilibrated, which generally requires 2 to 5 column volumes.

3.4 Recommended flow rate

- Depending on the type of chromatography column, a flow rate within the recommended flow rate range is generally selected, with the higher the column height the slower the flow rate. (See Table 2)

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 Wash

- Flush with equilibrium buffer until the UV absorption value drops to the appropriate value.

3.7 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, to collect different fractions, and to detect where the target is located.
- For more difficult to elute substances pure water can be used, or low concentrations of ethanol can be added to pure water as an eluent.

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 3: 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

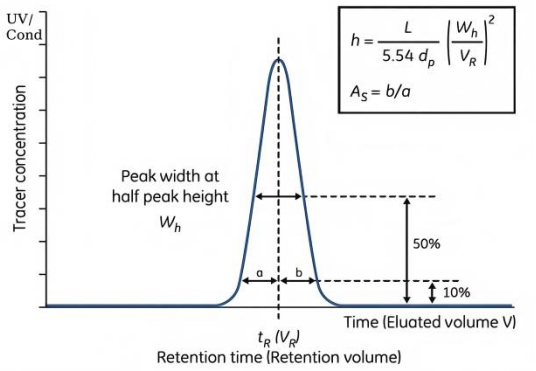
N=theoretical plate number

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)

In general, a value of HETP that is less than three times the average particle size of the medium and an asymmetry factor As between 0.8 and 1.8 indicates good column efficiency. (For TA-GF chromatography resin the number of plates per meter should be greater than 10,000). For unsatisfactory results, it is necessary to analyze the reasons and reload the column.

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 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.

5. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution does not have sterilization and de-thermogenic effect, it is recommended that TK-Col HIC-FF series preloaded columns can be treated with 1M NaOH for more

than 0.5-1h to achieve sterilization and de-thermogenic purpose.

TK-Col HIC-FF series preloaded columns are stored in 20% ethanol (or 10mM NaOH). To prevent ethanol volatilization and microbial growth, it is recommended that the columns be replaced with fresh 20% ethanol once every 3 months.

6. Destruction and recycling

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

7. Problems likely to be encountered

Table 4 :Problems, their causes and solutions

Problem	Cause analysis	Recommended Solutions
High column pressure	Clogged instrument filters	Cleaning of impurities at the filter, regular replacement of the filter discs
	Sample is sticky or too dirty	Filter membranes or dilute samples with an equilibrium solution
	Packing material not regenerated after last use	Perform CIP or replace with new packing
Purposeful samples appear in flow-through or wash	Low salt concentration in the equilibrium solution or weak target hydrophobicity	Increase the salt concentration in the buffer
	Aggregation of proteins or lipids in the medium	Timely and effective cleaning of media or replacement with new media
	overloaded	Selection of the sample volume is based on the selection of the appropriate load
The target protein is difficult to elute	Insufficient elution time	Reduced flow rate to prolong retention time of eluent
	The ionic strength of the eluent is too high	Reduce the ionic strength of the eluent appropriately
Purpose protein elution fraction is impure	The elution method is not suitable	Linear gradient elution followed by step gradient elution
	Fillers do not satisfy one-step removal	Addition of second-step chromatography (gel chromatography, affinity chromatography, ion exchange chromatography, etc.)

8. Ordering Information

Table 5: Goods number and packaging

Product	Item No.	Norm
TK-Col Butyl 4FF	Y6357	1×1ml
	Y6115	5×1ml
	Y6116	1×5ml
	Y6117	5×5ml
	Y611703	1×4.9ml
	Y611704	1×20ml
TK-Col Butyl-S FF	Y6358	1×1ml
	Y6118	5×1ml
	Y6119	1×5ml
	Y6120	5×5ml
	Y612003	1×4.9ml
	Y612004	1×20ml
TK-Col Octyl 4FF	Y6359	1×1ml
	Y6121	5×1ml
	Y6122	1×5ml
	Y6123	5×5ml
	Y612303	1×4.9ml
	Y612304	1×20ml
TK-Col Phenyl FF(LS)	Y6360	1×1ml
	Y6124	5×1ml
	Y6125	1×5ml
	Y6126	5×5ml
	Y612603	1×4.9ml
	Y612604	1×20ml
TK-Col Phenyl FF(HS)	Y6361	1×1ml
	Y6127	5×1ml
	Y6128	1×5ml
	Y6129	5×5ml
	Y612903	1×4.9ml
	Y612904	1×20ml