TK-Col Blue FF

TK-Col 16/10 Blue FF

TK-Col 26/10 Blue FF

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TA- Blue FF is an affinity medium with agarose as the base and Cibacron Blue 3GA as the ligand, which is physically and chemically stable, the ligand is not easy to be detached, has a long service life and has a wide range of applications. TA-Blue FF has been widely used in the separation and purification of various proteins, such as dehydrogenase, kinase, transferase, serum albumin, interferon and plasma proteins, etc. It can bind to proteins not only through specific interactions, but also through non-specific binding via charge and hydrophobicity.

TK-Col Blue FF pre-packed columns are ready-to-use affinity chromatography columns filled with TA-Blue FF media in TK-EC 1 ml ,TK-EC 4.9 ml ,TK-EC 5 ml ,TK-EC 20 ml chromatography vacutainer. TK-Col 16/10 Blue FF pre-packed columns are ready-to-use affinity columns filled with TA-Blue FF media in TK-EC 16/20 columns; TK-Col 26/10 Blue FF pre-packed columns are ready-to-use affinity columns filled with TA-Blue FF 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 pre-packed 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 albumin fusion proteins, dehydrogenases and other biomolecules. It has the following features:

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

2. Technical parameters

Table 1 TA-Blue FF Technical Parameters

Appearance	Blue slurry, layered on placement
Base frame	6% highly cross-linked agarose
Particle size distribution range	45~165μm
Functional groups	Cibacron Blue 3GA
Ligand density	~7.3μmol chromophore/mL filler
Binding capacity	>18mg HSA/mL filler
Pressure resistance	0.3 MPa
pH stability	4~12 (long term), 3~13 (short term)
Chemical stability	Stable in all commonly used water-soluble buffers: 8M urea, 6M guanidine hydrochloride, 70% ethanol
Temperature resistance	4~40°C, cannot be frozen, resistant to 121°C autoclaving
Storage	2~8°C, 20% ethanol or 2% benzyl alcohol, 0.1M KH2PO4 pH 8.0
Recommended Flow Rate	30-300cm/h

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 Blue FF	TA-Blue FF	1	7×25	0.2-2.0	2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments).	0.3MPa（3bar）	10
		4.9	8×100	0.2-2.5			
		5	16×25	1.0-10.0			
		20	16×100	1.0-10.0			
TK-Col 16/10 Blue FF		19.1-21.1	16×100（±5）	2.0-10.0			
TK-Col 26/10 Blue FF		50.4-55.7	26×100（±5）	3.0-26.0			

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: Binding buffer with low pH leads to enhanced protein binding, pH range between 5.5-8.5 is appropriate.
- Equilibration buffers with low ionic strengths enable enhanced protein binding, and concentrations between 5-50 mmol/L are appropriate.
- The presence of metal ions also leads to enhanced protein binding, and adding 0.1-10 mmol/L of metal ions (e.g., Mg2+, Ca2+, Zn2+, Mn2+, Cu2+, Co2+, Fe3+, and Al3+) to the buffer can enhance protein binding.
- Use the recommended flow rate to flush the column with the equilibration buffer. The pH and conductance of the buffer to be exported are consistent with that of the buffer prior to entering the column, which indicates that 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. (See Table 2)

3.5 Sampling

- Sample and sample volume: For complex protein mixed samples, the volume of the sample should not be too large, the larger the volume of the sample, the weaker the binding ability; but for the samples that bind to the media ligand exclusively do not need to give too much consideration to the volume of the sample.
- The concentration of the sample should not be too large, high concentration (more than 30mg/ml) may cause fluctuations in pH and ionic strength, resulting in the loss of protein activity, generally use binding buffer to dilute the sample.
- The viscosity of the sample needs to be appropriate, high viscosity samples can cause irregular flow rates during the chromatography process.
- In order to avoid clogging the chromatography column will reduce the resolution efficiency and service life of the column, so the sample solution needs to be centrifuged or filtered with a 0.45 μm filter before the sample is loaded.

3.6 Rinse

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

3.7 Eluent

- To optimize the elution conditions, change the ionic strength of the elution buffer, by increasing the molar concentration of the buffer or by adding salt (KCl and NaCl) concentrations.
- Change the pH of the elution buffer.
- Change the polarity of the elution buffer, e.g., by adding 50% ethylene glycol, 10% dioxane, or other organic solvents.
- 3 times the column volume of equilibration buffer containing appropriate concentrations of specific ligands such as enzyme substrates, enzyme substrate generators, cofactors, inhibitors and activators.

3.8 Regeneration and rebalancing

- Regeneration; rinse the chromatography column with purified water or 30% isopropanol (70% ethanol).
- Re-equilibration: after rinsing with equilibration buffer it is ready for the 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

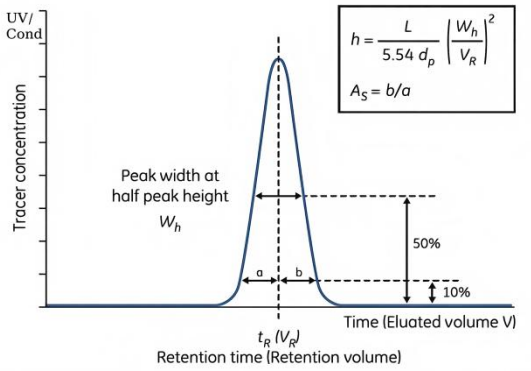
N=theoretical plate number

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)

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).

- Denatured proteins: Wash 3-4 column volumes with 0.1M NaOH, followed by 3-4 column volumes of 70% ethanol or 2M potassium thiocyanate; or 2-3 column volumes of 6M guanidine hydrochloride; rinse immediately with at least 5 column volumes of equilibration buffer.
- Strongly hydrophobic substances or lipids: Wash the column with 2-4 column volumes of 70% ethanol or 30% isopropanol and rinse immediately with at least 5 column volumes of equilibration buffer.

5. Sterilization and storage

Since the preservation solution 20% ethanol or 2% benzyl alcohol (pH 8.0) containing 0.1M KH_2PO_4 is not bactericidal or pyrogenic, it is recommended that TA-Blue FF media can be treated with 70% ethanol for 12h to reduce the risk of microbial contamination prior to and during use, or sterilized by sterilizing the medium in purified water at 121°C for 15min to achieve the sterilization purpose.

TA-Blue FF is sold in a preservation solution of 20% ethanol or 2% benzyl alcohol (pH 8.0) containing 0.1M KH_2PO_4. After use, store TA-Blue FF in 20% ethanol (pH 8.0) containing 0.1M KH_2PO_4 in a sealed container at 2-8°C. It is recommended that the preservation solution be replaced with a fresh one every 3 months to prevent ethanol evaporation and microbial growth.

6. Destruction and recycling

Since TA-Blue FF is difficult to degrade in nature, incineration is recommended for environmental protection.

7. Ordering Information

Table 4 Article number and packaging

Product	Item No.	Norm
TK-Col Blue FF	Y6279	1×1ml
	Y6281	5×1ml
	Y6280	1×5ml
	Y6282	5×5ml
	Y628203	1×4.9ml
	Y628204	1×20ml
TK-Col 16/10 Blue FF	Y6283	1pac.
TK-Col 26/10 Blue FF	Y6284	1pac.