TK-Col Protein G 4FF

TK-Col 16/10 Protein G 4FF

TK-Col 26/10 Protein G 4FF

Affinity Chromatography Prepacked Columns

Product Manual

Content

1. Product Introduction

TA-Protein G 4FF is an affinity medium made by immobilizing Protein G onto a 4FF framework by cyanogen bromide activation.Protein G has a broader binding spectrum than Protein A. Protein G binds strongly to the Fc fragment of an antibody and weakly interacts with the Fab fragment of an antibody, so TA-Protein G 4FF is commonly used for the isolation and purification of antibodies from cell cultures or antibody fragments, as well as purification of antibodies from sera of various species. Protein G has a broader binding spectrum than Protein A. Protein G binds strongly to the Fc fragment of the antibody and weakly interacts with the Fab fragment of the antibody, so TA-Protein G 4FF is commonly used for the isolation and purification of antibodies or antibody fragments from cell cultures, as well as for the purification of antibodies from sera of various species.

TK-Col Protein G 4FF preloaded columns are ready-to-use affinity chromatography columns filled with TA-Protein G 4FF media in TK-EC 1ml ,TK-EC 4.9 ml ,TK-EC 5ml ,TK-EC 20ml chromatographic vacutainers; TK-Col 16/10 Protein G 4FF preloaded columns are ready-to-use affinity chromatography columns packed with TA TK-Col 16/10 Protein G 4FF preloaded columns are ready-to-use affinity chromatography columns filled with TA-Protein G 4FF media in TK-EC 16/20 chromatography vacutainer columns; TK-Col 26/10 Protein G 4FF preloaded columns are ready-to-use affinity chromatography columns filled with TA-Protein G 4FF media in TK-EC 26/20 chromatography vacutainer 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: TA-Protein G 4FF technical parameters

Appearance	White slurry, layered on placement
Base frame	Highly cross-linked 4% agarose
Particle size distribution range	45-165μm
Functional groups	Protein G (E.coli)
Ligand density	~2mg Protein G/mL media
Crosslinking mode	Cyanogen bromide activation
Dynamic binding load (20 mM PB, pH 7.0, H=10 cm)	~20mg Human IgG/mL media
Chemical Stability	40°C for 1 week: 1 M HAc (pH 2.0), 20 mM sodium phosphate, 1% SDS, 6 M hydrochloric acid guanidine, 70% ethanol; Room temperature 2h: 0.1 M HCl (pH1.0), 8 M urea (pH10.5)
Pressure resistance	0.3MPa
Flow rate range	150-250cm/h
pH Stability	3-9 (working), 2-10 (CIP)
Storage	2~8℃, 20% ethanol or 2% benzyl alcohol

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 Protein G 4FF	TA-Protein G 4FF	1	7×25	<1.5	2-8°C, 20% ethanol or 2% benzyl alcohol (for international shipments).	0.3MPa （3bar）	10
		4.9	8×100	<2			
		5	16×25	<8			
		20	16×100	<8			
TK-Col 16/10 Protein G 4FF		19.1-21.1	16×100 （±5）	3-8			
TK-Col 26/10 Protein G 4FF		50.4-55.7	26×100 （±5）	8-22			

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:**Protein G binds to different types of IgG in a neutral environment and 20 mM PB, pH 7.0 is recommended as a binding buffer.
- 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.
- A comparison of the binding capacities of Protein A and Protein G and different genera of immunoglobulins (Table 3) is shown below.

Table 3 Comparison of binding strength of Protein A and Protein G to different types of immunoglobulins

Category	Subclass	Protein G binding intensity	Protein A binding intensity
Human	IgA	-*	variable
	IgD	-	-
	IgE	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	++++	-
	IgG_4	++++	++++
	IgM	-	variable
Poultry egg yolk	IgY	-	-
Cow		++++	++
Dog		+	++
Sheep		++	-
Guinea pigs	IgG_1	++	++++
Large-cheeked rodents		++	+
Horses		++++	++
Tree kangaroo		+	-
Camels		+	-
Rhesus monkey		++++	++++
Mice	IgG_1	++++	+
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	+++	++
	IgM	-	variable
pig		+++	+++
Rabbit		+++	++++
Rat	IgG_1	+	-
	IgG_{2a}	++++	-
	IgG_{2b}	++	-
	IgG_3	++	+
Sheep		++	+/-

*More plus signs indicate stronger binding, while minus signs indicate very weak or even no binding.

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 usually change the pH of the buffer for elution, and it is recommended to use 0.1 M glycine, pH 2.5- 3.0, as the elution buffer. In order to maintain the activity of some acid-sensitive IgG, 1/10 of the collection volume of 1 M Tris-HCl, pH 9.0, can be pre-added to the elution collection tube for neutralization.

3.8 Regeneration and rebalancing

- **Regeneration:** Immediately after elution, the media should be washed with 2 to 3 column volumes of elution buffer and then equilibrated with 2 to 3 column volumes of binding buffer. If substances such as denatured proteins or lipids from the process cannot be eluted during regeneration, they can be removed by in-situ washing operation.
- **Re-equilibration:** rinsing with the equilibration buffer is sufficient for a second up-sampling, 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

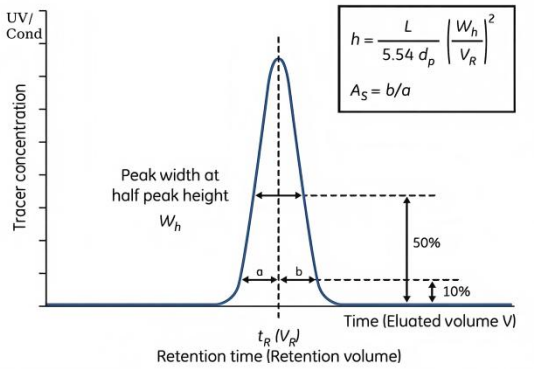
N=theoretical plate number

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).

- Removal of lipoproteins and lipids: soak in 70% ethanol for 12h, then wash with at least 5 column volumes of binding buffer.
- Removal of strongly hydrophobic proteins, precipitated proteins: can be contacted with a non-ionic detergent, such as 0.1% Trtion X-100 at 37℃ for 1min, then quickly washed with at least 5 column volumes of binding buffer.

5. Sterilization and storage

TK-Col/TK-Col 16/10/TK-Col 26/10 Protein G 4FF pre-packed columns can be sterilized by treatment with 70% ethanol for more than 12h.

TK-Col/TK-Col 16/10/TK-Col 26/10 Protein G 4FF pre-packed columns are stored in 20% ethanol at 2-8°C. To prevent ethanol volatilization and microbial growth, it is recommended that the columns be replaced with fresh preservation solution once every 3 months after use.

6. Destruction and recycling

Since the packing material in the TK-Col Protein G 4FF 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 Protein G 4FF	Y6201	1×1ml
	Y6202	5×1ml
	Y6203	1×5ml
	Y6204	5×5ml
	Y620403	1×4.9ml
	Y620404	1×20ml
TK-Col 16/10 Protein G 4FF	Y6205	1pac.
TK-Col 26/10 Protein G 4FF	Y6206	1pac.