TA-Protein G 4FF

Affinity Chromatography Resin

Product Manual

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Content

1. Product Introduction

TA-Protein G 4FF is an affinity medium made by immobilizing Protein G onto TA-4FF by cyanogen bromide activation.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. 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.

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	~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, 0.1MPa, TK-EC50, H=25cm
pH Stability	3-9 (working), 2-10 (CIP)
Storage	2~8°C, 20% ethanol or 2% benzyl alcohol

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 TA-Protein G 4FF needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.15 (i.e., compression ratio of approximately 1.15)

  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 it with about 3 times the volume of the loading solution (20% ethanol), (when the volume is relatively large or the conditions are not available, you can also use to wait for the gel to be stratified, then draw off the upper layer of the solution, then add the 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: Transfer the cleaned medium from the funnel to a beaker or other suitable container, add the column mounting solution until the concentration of the gel suspension is 45~55%, 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 column loading flow rate to 45cm/h, open the bottom valve/plug of the chromatography column, turn on the flow rate, use the set flow rate to press until the gel surface is clear and stable, mark the position of the gel surface when it is stable.

● Wait for the gel suspension to finish settling needs to be kept for another 10min or more, mark the position of the gel surface, and then stop the pump.

● Remove the column loader (if any), install the upper column head, lower the column head to a position of about 0.5cm on the gel surface, continue to press the gel once at a flow rate of 160cm/h, mark the position of the gel surface.

● Stop the pump, slightly relax the sealing ring of the column head, 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 0.3cm below the marked position, tighten the sealing ring of the column head, close the valve at the bottom, and the loading of the column 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

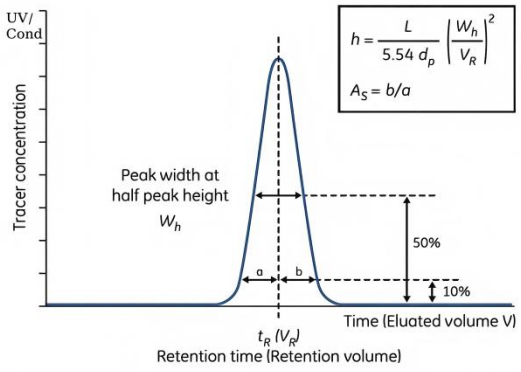
N=theoretical plate number

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:** 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.
- 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.

- **Flow rate:** According to the height of the column generally choose 50-300cm/h flow rate, the higher the column height the slower the flow rate, reduce the flow rate to increase the contact time of the sample and the medium can improve the binding load.
- **Sample Preparation:** To prevent the sample from clogging the column, the sample needs to be filtered through a 0.45 μm microporous membrane and the pH and conductivity of the sample adjusted to match that of the Equilibration Buffer before loading.
- **Equilibration:** Wash the column with equilibration buffer until the UV absorption drops to an appropriate value.
- **Sampling:** Sample the prepared sample solution according to the set conditions.
- **Wash:** The column is washed with binding buffer until the UV absorption drops to an appropriate value.
- **Elution:** Usually change the pH of the buffer for elution, it is recommended to use 0.1M glycine pH2.5~3.0 as elution buffer. In order to maintain the activity of some acid-sensitive IgGs, the elution collection tube can be pre-loaded with 1/10 of the collection volume of 1M Tris-HCl, pH 9.0 for neutralization.
- **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.

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).

- 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.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservative is not bactericidal or de-pyrogenic, it is recommended that TA-Protein G 4FF media can be treated with 70% ethanol for 12h before and during use to reduce the risk of microbial contamination.

TA-Protein G 4FF 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°C in an airtight 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 TA-Protein G 4FF 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
TA-Protein G 4FF	Y5008	25mL
	Y5009	100mL
	Y5010	500mL
	Y5011	1L
	Y5012	5L
	Y5013	10L
	Y5014	20L