TA-Blue FF

Affinity Chromatography Resin

Product Manual

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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.

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

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-Blue FF 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:Shake the media suspension well and measure the volume calculated by the above method,

pour it into a funnel, draw off the liquid and wash it with about 3mL of purified water/mL of media, repeat the washing 3 times, each time when adding the washing liquid, you need to use a glass rod or stirring stick to stir, in order to wash off the original preservation liquid better.

- 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 50~75%, 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 flow rate (TA-Blue FF can be set to 75cm/h for a loading height of 15~20cm), open the bottom valve/plug of the chromatography column, turn on the flow rate, and use the set flow rate to press until the adhesive surface is clear and stable, and mark the position of the adhesive surface when it stabilizes.
- Remove the column loader (if any), install the upper column head, lower the column head to about 0.5cm above the gel surface, set the flow rate to 260cm/h and continue to press the column until the gel surface is clear and stable, mark the column height when the gel surface is stable.
- Stop the pump, open the valve/plug on the column head, close the valve/plug at the bottom of the column, slightly relax the sealing ring of the column head, press down the column head to about 0.3cm below the marked position, tighten the sealing ring of the column head, close the valve/plug at the column head, and the installation 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

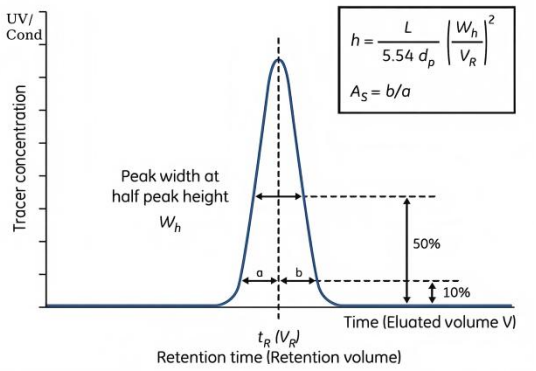
N=theoretical plate number

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

- **Samples:** preferred phosphate buffer, pH neutral to weakly alkaline (7~8), avoid EDTA and citrate, etc. Tris-HCl can also be used, but should be avoided in the case of metal ions and protein affinity is very weak.Commonly added reagents and concentrations that do not affect and affect metal chelation chromatography are listed in Tables 3 and 4, respectively.
- For complex protein mixture samples, the concentration of the sample should not be too low, the lower the binding ability is the weaker; however, for samples that bind specifically to the medium ligand, the sample concentration does not need to be considered too much.
- The concentration of the sample should not be too large, high concentration (more than 30mg/mL) may cause fluctuations in pH and ionic strength, affecting the binding, high concentration can be used to dilute the sample with binding buffer.
- Pay attention to the viscosity of the sample, high viscosity samples can cause uneven flow rate during the chromatography process.
- The sample solution should be centrifuged or filtered through a 0.45μm filter before sampling to avoid clogging the chromatography column or reducing the resolution efficiency and service life of the column.
- **Binding buffer:**
- Binding buffers with low pH promote protein binding, and a pH range between 5.5-8.5 is generally appropriate.
- Equilibration buffers of low ionic strength promote protein binding, and a binding buffer concentration between 5-50 mmol/L is appropriate.
- The presence of metal ions also enhances protein binding, and the addition of 0.1-10 mmol/L of metal ions (e.g., Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Fe^{3+}, and Al^{3+}) to the buffer can enhance protein binding.

- **Flow rate:** According to the height of the column generally choose a flow rate of 150~300cm/h, the higher the column height the slower the flow rate.
- **Sample preparation:** To prevent the sample from clogging the column, the sample needs to be filtered with 0.45μm microporous filter membrane and the pH and conductivity of the sample should be adjusted to be consistent with that of the binding buffer before the sample is loaded, and the volume of the sample should be determined according to the content of the impurities in the sample and the binding loading of TA-Blue FF.
- **Equilibration:** Wash the chromatography column with equilibration buffer until the UV absorption value drops to an appropriate value.
- **Sample loading:** The prepared sample solution is loaded according to the set program.
- **Wash:** Wash with binding buffer until the UV absorption value drops to an appropriate value.
- **Elution:** One or more of the following can be used in conjunction to elute the bound protein

➢ Change the ionic strength of the elution buffer by increasing the concentration of salts (KCl and NaCl) in the buffer.

➢ Altering to increase 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.

➢ Addition of appropriate concentrations of specific ligands such as enzyme substrates, enzyme substrate generators, cofactors, inhibitors and activators.

- **Regeneration:** High pH (0.1M Tris, 0.5M NaCl, pH 8.5) and low pH (0.1M NaAc, 0.5M NaCl, pH 4.5) alternating cleaning methods can be used to remove strongly bound proteins to regenerate the column.

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).

➢ 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.

4. 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.

5. Destruction and recycling

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

6. Ordering Information

Table 5 Article number and packaging

Product	Item No.	Norm
TA- Blue FF	Y5092	25mL
	Y5093	100mL
	Y5094	500mL
	Y5095	1L
	Y5096	5L
	Y5097	10L
	Y5098	20L