TA-Benzamidine 4FF(HS) Affinity Chromatography Resin

Product Manual

楚天微球生物技术(长沙)有限公司
TRUKING MICRO-SPHERE BIOTECHNOLOGY (CHANGSHA) CO., LTD

Content

1. Product Introduction

TA-Benzamidine 4FF (HS) is an affinity chromatography medium made by coupling p-aminobenzenecarboximidamide to agarose gel TA-4FF, which is commonly used for the isolation and purification of serine proteases or the removal of serine proteases from biological samples. Benzamidine analogs are broad-spectrum inhibitors of serine proteases (e.g., trypsin, thrombin, urokinase, kinin-releasing enzyme, and prokinin-releasing enzyme), and can be used as ligands for the purification of such substances.

Table 1 TA-Benzamidine 4FF (HS) technical parameters

Appearance	White slurry, layered on placement
Base Frame	4% highly cross-linked agarose
Average particle size	90μm
Functional groups	p-Aminobenzamidine
Ligand density	≥12μmol/mL filler
Binding load	≥35mg trypsin/mL filler
Pressure resistance	0.3 MPa
Chemical stability	Common aqueous solutions: 8M urea, 6M guanidine hydrochloride, pH=1,2,3,4 Hydrochloric acid solution, 0.025M borax solution pH=8, 9, 10, 11
pH stability	2~8 (working); 1~9 (CIP)
Storage	2~8°C, 20% ethanol or 2% benzyl alcohol, 50 mM NaAc pH 4.0
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-Benzamidine 4FF (HS) 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.

● Wash the medium: Shake the medium 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 column filling solution /mL of the medium, repeat the washing 3 times, each time you add the washing solution, you need to use a glass rod or stirring stick to stir in order to wash off the original preservation solution better.

● Gel suspension preparation: Transfer the cleaned medium from the funnel to a beaker or other suitable container, add the column filling solution to a concentration of 50~75% of the gel suspension, 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 (using a column loader if necessary), taking care not to introduce 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).

● When the column height is 15cm, the loading pressure can be set to 0.18MPa, open the bottom valve/plug of the chromatography column, start the pump, and use the set flow rate to press until the gel surface is clear and stable, and mark the position when the gel surface is stable.

● Remove the column loader (if there is one) and lower the column head to a position about 0.5 cm above the glue surface, continue to press the column at the pressure described above until the glue surface is clear and stable, marking the position of the glue surface.

● 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

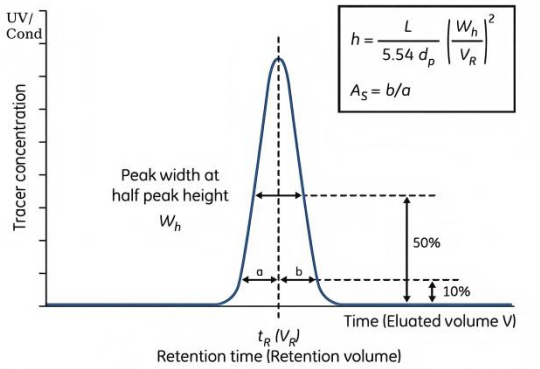
N=theoretical plate number

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

- **Sample:** To avoid clogging the chromatography column, the sample solution needs to be centrifuged or filtered through a 0.45 μm filter before loading. The viscosity of the sample needs to be appropriate, high viscosity samples will cause uneven flow rate during the chromatography process and affect the binding efficiency.
- **Binding buffer:** neutral buffer is generally used, such as 50 mM Tris, 0.5 M NaCl, pH 7.4.
- **Flow rate:** 30~300cm/h flow rate is recommended.
- **Sample preparation:** adjust the pH and conductivity of the sample to be consistent with the equilibrium buffer, and determine the volume of the sample according to the impurity content in the sample and flow rate.
- **Sample loading:** the prepared sample is loaded according to the set conditions.
- **Wash:** Wash with binding buffer until the UV absorption value drops to an appropriate value.
- **Elution mode 1:** commonly used to reduce the pH for elution, e.g., 50 mM glycine, pH 3.0. Collected elution is immediately neutralized with 1M Tris, pH 9, and 1mL elution requires 60~200μl 1M Tris.
- **Elution Mode 2:** Optionally, competitive elution with the addition of p-aminobenzenecarboximidamide is also possible, e.g. 50 mM Tris, 0.5 M NaCl, 20 mM p-aminobenzenecarboximidamide, pH 7.4.
- **Regeneration:** 2 column volumes of high pH buffer (0.1MTris-HCl, 0.5M NaCl, pH 8.5) and low pH buffer (0.1M sodium acetate, 0.5M NaCl, pH 3) were washed three times alternately; 10 column volumes of binding buffer equilibrated the chromatography column.

3. Cleaning and regeneration

As the number of times the chromatography medium is used increases, so does the accumulation of

contaminants on the column. Regular in-situ cleaning prevents the accumulation of contaminants and maintains a stable working condition. Customers can determine the frequency of in-situ cleaning according to the degree of media contamination during use (if contamination is severe, it is recommended that in-situ cleaning be performed after each use to ensure reproducible results).

Recommended cleaning conditions for different types of impurities and contaminants are as follows:

- **Wash of precipitated or denatured material:** Wash with 2 column volumes of 6M guanidine hydrochloride followed by 5 column volumes of equilibration buffer.
- **Wash of hydrophobically bound material:** 2 column volumes of 70% ethanol followed by 5 column volumes of equilibration buffer.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol (pH 4.0) preservation solution containing 50 mM sodium acetate is not bacteriostatic or pyrogenic, it is recommended that TA-Benzamidine 4FF(HS) media can be treated with 20% ethanol containing 0.1 mM acetic acid for more than 12 h prior to and during use to minimize the risk of microbial contamination.

TA-Benzamidine 4FF(HS) is sold with 20% ethanol containing 50 mM sodium acetate or 2% benzyl alcohol (pH 4.0) as a preservation solution. After use, TA-Benzamidine 4FF(HS) should be stored in a 20% ethanol (pH 4.0) solution containing 50 mM sodium acetate and kept 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-Benzamidine 4FF(HS) is difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TA-Benzamidine 4FF(HS)	Y5134	25mL
	Y5135	100mL
	Y5136	500mL
	Y5137	1L
	Y5138	5L
	Y5139	10L
	Y5140	20L