

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Benzamidine Focurose 4FF (HS) (Benzamidine-4FF (HS))

Catalog No: E-CM-AF13

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Product introduction

Benzamidine-4FF (HS) is suitable for separation and purification of trypsin, thrombin, enterokinase, urokinase, and prekallikrein. It can also be used for rapid removal of cell culture supernatant, bacteria lysate, and serine proteases in serum.

Advantages

1. Quick and easy (one-step purification).
2. High loading capacity.
3. Easy to be implemented.

Performance index

Ligand	p-aminobenzamidine [pABA]
Matrix	Highly cross-linked 4% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Binding capacity	25-50 mg (Trypsase)/mL (media)
pH stability	1-9 (short-term) 2-8 (long-term) 2-8 (application)
Chemical stability	All of the common buffers, 8M Urea, 6M Guanidine hydrochloride
Flow rate	300-700 cm/h (0.3MPa, XK16/40. Column bed height: 30 cm)
Pressure	$\leq 0.3\text{MPa}$
Storage buffer	0.05M Acetate buffer, 20% Ethanol, pH 4.0
Storage temperature	4~8 $^{\circ}\text{C}$

Operation (take 1 mL column and 5 mL column for example)

1. Wash (water)

Wash the media with 5~10 CV (column volume) of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This operation is used to remove the 20% ethanol in media.

2. Equilibration

Balance the media with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) until the baseline turns stable and then set to zero.

Note: This procedure is used to balance the media. Make sure that the pH and component in media are in accordance with sample.

3. Sample application

Apply the sample with flow rate of 0.2 mL/min (1 mL) or 1.0 mL/min (5 mL) after centrifugation and filtration. Wash with the equilibrium liquid until the baseline tends to zero.

Note: Benzamidine-4FF (HS) has certain ion-effect. Make sure that samples containing 0.5M NaCl at least, and the pH of sample solution should be maintained to 7.4-8.0.

4. Elution

Elute with 5~10 CV of eluent at with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Note: Low flow rate may increase the concentration of target protein in the eluted solution.

5. Wash with water

Wash the media with 5~10 CV of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This procedure is used to remove the eluent in media.

6. Storage

Wash the media with 5~10 CV of 20% ethanol with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and the store the media.

Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30°C (4~8°C is preferred).

7. Preparation of buffer

Equilibrium liquid: 0.05M Tris-HCl, 0.5M NaCl, adjust the pH to 7.4. Store the prepared equilibrium liquid at room temperature.

Note: The pH of prepared equilibrium liquid must be ensured to be 7.3. If the pH of sample solution <6.5 or pH>8.0, the reaction between sample and media is weak or even invalid.

Eluent:

Eluent I : 0.05M Glycine, adjust the pH to 3.0. Store the prepared eluent at 4°C (recommended).

Eluent II : 0.01M HCl, 0.5 M NaCl, adjust the pH to 2.0. Store the prepared eluent at 4°C (use the Eluent II while the elution effect is poor).

Eluent III: 0.05M Tris-HCl, 0.5M NaCl, 0.02M p-amino-benzamidine, adjust the pH to 7.4. (This elution method can be applied when the target is sensitive to pH. p-amino-benzamidine has a high absorbance value at 280 nm, thus the components of eluent must be determined by SDS-PAGE or other detection methods.)

Note: Prepare fresh eluent before use. Store the prepared eluent at 4°C (Vulnerable to microbial growth).

Cleaning

The excellent performance of media (e.g. loading ability, mobility, column efficiency, etc.) can be recovered after cleaning the strong coupling substance (e.g. some strong coupling protein, denatured protein, lipids, etc.).

It is recommended to wash the media after used for each 5 times. The exact washing frequency should be adjusted according to the cleanliness of the purified sample.

1. Wash the media with 2~5 CV of 1.0% Triton X-100 or 70% ethanol, then wash the media with 5~10 CV of purified water immediately.

Note: This procedure is used to remove the hydrophobic binding substances.

2. Wash the media with 2~5 CV of 6M guanidine hydrochloride, then wash the media with 5~10 CV of purified water immediately.

Note: This procedure is used to remove the precipitates or denatured substances accumulated in the media.

3. Store the media after washed with 5~10 CV of 20% ethanol.

Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30°C (4~8°C is preferred).

Troubleshootings

Problem	Possible cause	Suggestion
The target compound does not combine with the media or the combining amount is low when purifying	Overloading of sample volume.	Decrease the sample volume.
	Speed of sample loading is too fast.	Reduce the flow speed of sample loading.
	Protein or lipids accumulate in the media.	Wash the media timely and effectively.
	pH of sample or equilibrium liquid is not in the right range.	Make sure that the pH of sample or equilibrium liquid is between 6.5~8.0.
No target compound was collected or only a small amount of target compound was collected	The target compound does not combine with the media or the combining amount is low.	Confirm whether the target combine with media or not.
	Unsuitable elution condition.	Increase the GSH concentration to 20-40 mM and make sure that the pH of eluent is between 8.0~9.0
	Insufficient elution time.	Decrease the flow rate and prolong the retention time of eluent.
	Insufficient elution volume.	Increase the elution volume.
	The target compound accumulates in the elution buffer.	Check the stability of target compound in the elution buffer (salt concentration, pH, etc.)
Low purity of target compound	Sample has not been pretreated.	Samples must be centrifuged or filtered before loading.
	High viscosity of sample.	Dilute the sample properly with equilibrium liquid to decrease the viscosity.
	Insufficient washing.	Increase the washing volume until the baseline smooth and keep consistence with equilibrium liquid.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	Poor elution condition, fast elution speed and abrupt elution gradient.	Adjust the elution condition.
	The target compound degrades.	Determine the stability of target compound.

	Bad loading effect of column resin.	Reload or re-purchase.
	Non-specificity absorption of impurity materials.	Add appropriate additive to reduce the non-specific absorption.
	The top of separation column has a large volume of sample.	Reload the column or reduce the volume of the sample.
	There is microbial grow in the media.	Correctly store the media after used.
Decrease of loading volume	Speed of sample loading is too fast.	Reduce the flow speed of sample loading.
	Protein or lipids accumulate in the media.	Wash the media timely.
	Ligand dropped off due to excessive use.	Re-couple with new media
The chromatographic peak rises slowly	The media was loaded too tight.	Reload the column.
The chromatographic peak trails	The media was loaded too loose.	Reload the column.
The column bed cracks or being dry	Leakage occurred or a large volume of bubbles was introduced.	Check whether there is leakage or bubble, reload the column.
Flow of the column is exceedingly slow	Protein or lipids accumulate in the media.	Wash the media or filter membrane timely.
	Protein precipitates in the media.	Adjust the content of equilibrium liquid and wash buffer to maintain the stability of target compound and combining efficiency of media.
	There is microbial grow in the media.	Filter and degas all the reagents. Samples must be centrifuged or filtered before loading.