Elabscience®

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CNBr Focurose 4FF

Catalog No: E-CM-AF17

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.

Please read this manual carefully before use to ensure the performance and successful operation. If you have any questions, please contact our Technical Support.

Product introduction

CNBr Focurose 4FF is a kind of fast flow purification media which has been activated with cyanogen bromide (CNBr). It is suitable for coupling of protein, polypeptide, nucleic acids and other biomolecules containing amino groups. CNBr Focurose 4FF has been verified repeatedly and widely used in the bio-pharmaceutical purification procedure.

Advantages

1. Wide application range: It can be used for coupling of macro biomolecules containing amino groups.
2. Multi-point coupling. Simple, flexible, fast and effective. It can efficiently maintain the biological activity and stability of biomolecules.
3. Fast flow rate, high yields and easy to be implemented.

Performance index

Matrix	Highly cross-linked 4% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Combined capacity	30 mg (Trypsinogen)/mL (media)
pH stability	2-14 (short-term) 3-13 (long-term)
Maximum flow rate	700 cm/h
Pressure	≤ 0.3 MPa
Storage buffer	100% Acetone
Storage temperature	4~8℃

Coupling condition

1. Wash (Solution A)

Take appropriate amount of settled gel (0.83g, equal to about 0.1 mL after washed), resuspend the media with 5 times volume of Solution A. Drain the liquid after 5 min. Repeat this procedure for 5 times.

Notes: This operation is used for activation of media. Make sure that the wash volume of Solution A is sufficient and the wash time is about 30 min (the groups on the media will hydrolyze if washed too long).

2. Preparation of ligand solution

Dissolve the target coupled biomolecule with Solution B or transposition the replace the biomolecule in Solution B (the concentration of biomolecule should be 1~10 mg/mL, the recommended concentration is 5 mg/mL).

Note: Ensure that the pH and salt concentration during coupling is in accordance with Solution B.

3. Coupling

Mix the washed media and prepared sample at the ratio of 1:1 (volume: volume). Mix fully and gently for 3~4 hours at room temperature. Drain the solution after coupling successful (by detecting the concentrations of biomolecule before and after coupling).

Note: It is recommended to operate the coupling for 3~4 hours at room temperature or overnight at 4~8℃ for unstable ligands.

4. Wash (Solution C)

Resuspend the coupled media with 5 times volume of Solution C. Drain the liquid after 5 min. Repeat this procedure for 3 times.

Note: This procedure is used for washing the residual biomolecule in the media. Washing procedure must be sufficient.

5. Blocking

Resuspend with 5 times volume of Solution C. Mix fully and gently for 3~4 hours at room temperature. Drain the liquid.

Note: This procedure is used for blocking groups on the media.

6. Wash (Solution D and Solution E)

Resuspend the blocked media with 5 times volume of Solution D. Drain the liquid after 5 min. Then resuspend the media with 5 times volume of Solution E. Drain the liquid after 5 min. Repeat this procedure for 3 times.

Note: This procedure is used to remove the biomolecules which are defective tightness coupled.

7. Storage

Resuspend the media with 5 times volume of purified water and drain the liquid. Then resuspend the media with 5 times volume of 20% ethanol and drain the liquid. Store the media by immersing with 20% ethanol.

Note: This procedure is used to store the media and avoid of microorganism.

8. Preparation of solution

Solution A: 0.001M HCl, 0.5M NaCl, pH 3.0. Store at 4~8℃. (Solution A should be pre-cooled before used.)

Solution B: 0.2M $NaHCO_3$, 0.5 M NaCl, pH 8.3 (pH=8.5-9.0 if the coupled biomolecule is IgG.) Store at room temperature.

Solution C: 0.1M Tris-HCl, pH 8.3. Store at room temperature.

Solution D: 0.05M Tris-HCl, 0.5M NaCl, pH 8.5. Store at room temperature.

Solution E: 0.05M Glycine, 0.5M NaCl, pH 3.5. Store at room temperature.

Solution F: 1.0M NaCl. Store at room temperature.

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. Conventional cleaning
   (1) Wash the media with 5~10 column volumes of purified water.
   (2) Wash the media with 5~10 column volumes of Solution D.
   (3) Wash the media with 5~10 column volumes of Solution E.
   (4) Wash the media with 5~10 column volumes of Solution F.
   (5) Wash the media with 5~10 column volumes of purified water.
   (6) Store the media after washed with 5~10 column volumes of 20% ethanol.

2. Deep cleaning
   (1) Wash the media with 2~5 column volumes of 0.2% non-ionic detergent, then wash the media with 5~10 column volumes of purified water immediately.
   (2) Wash the media with 2~5 column volumes of 6M guanidine hydrochloride, then wash the media with 5~10 column volumes of purified water immediately.
   (3) Store the media after washed with 5~10 column volumes of 20% ethanol.

Note: Whether deep cleaning is suitable depends on the stability of coupling biomolecules. A pre-experiment is recommended to be operated before deep cleaning to determine the stability of biomolecules.

Trouble shootings

Problem	Possible cause	Suggestion
Low coupling efficiency	Salt concentration or pH Solution B is wrong.	Check whether the preparation of Solution B is right.
	Insufficient coupling time.	Prolong the coupling time
	Unsuitable pre-activating resin.	Try other kinds of pre-activating resin.
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.
	Sample inactivates in the process of storage or sample loading.	Store the sample to be purified correctly to maintain the activity.
	Low combining ratio between ligand and target compound.	Try to increase the ligand concentration during coupling.
	Ligand degrades during coupling or washing.	Determine the stability of ligand during coupling or washing.
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.	Reduce the flow speed of sample loading and check the combining ability of media.
	Unsuitable elution condition.	Change the corresponding elution condition or increase the elute ability of elution buffer.
	The target compound accumulates in the elution buffer.	Check the stability of target compound in the wash 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	Flow rate of sample application is too fast.	Reduce the flow rate 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.
	Sample inactivates in the process of storage or sample application.	Store the sample to be purified correctly to maintain the activity.
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 applied.