Elabscience®

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EAH Focurose 6FF (EAH-6FF)

Catalog No: E-CM-AF14

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

EAH-6FF is a kind of fast flow purification media which was prepared by covalently coupling the ethylenediamine to agarose matrix. It contains an amino group at the end of the spacer, and is mainly suitable for coupling of small molecular compounds (ligand) containing free carboxyl.

The coupling should be reacted under acidic conditions (acid catalysis). Addition of coupling reagent (carbodiimide, such as EDC, CMC) can promote the condensation of free amino and free carboxyl.

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 6% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Active group	Amino
Ligand density*	0.4-0.5 mM Cl^-/mL (media)
Spacer arm	15 atoms
Coupling temperature	4-25℃
Coupling pH	4.5-6.5
pH stability	3-14
Chemical stability	All commonly used buffers
Flow rate	250-600 cm/h (0.3MPa, XK16/40. Column bed height: 30 cm)
Storage buffer	20% Ethanol containing 0.9% NaCl
Storage temperature	4~8℃

*: The ligand density depends on the type of ligand and coupling condition.

Coupling condition

1. Wash (Solution A)

Take appropriate amount of settled gel, resuspend the media with 5 times volume of A Solution. Drain the liquid after 3 min. Repeat this procedure for 3 times.

Notes: This operation is used for activation of media. Make sure that the wash volume of A Solution is sufficient.

2. Wash (Solution B)

Resuspend the media with 20 times volume of B Solution. Drain the liquid after 3 min. Repeat this procedure for 3 times.

Notes: This operation is used for activation of media. Make sure that the wash volume of A Solution is sufficient.

3. Preparation of ligand solution

Dissolve appropriate amount of small molecular substances to be coupled with Solution A. Adjust the pH of dissolved solution to be in accordance with Solution A.

Note: Ensure that the pH and salt concentration during coupling is in accordance with Solution A. The amount of small molecular substances should be confirmed after optimized with experiment.

4. Coupling

Mix the washed media and prepared sample at 1:2 (volume: volume). Add appropriate amount of coupling reagent and adjust the pH to be in accordance with A Solution.

Mix gently and fully for 2~24 hours at 4℃ or room temperature. Drain the solution after coupling successful (by detecting the concentrations of biomolecule before and after coupling).

Note: It is recommended to adjust the pH of mixture to be in accordance with Solution A by adding NaOH or HCl in the coupling procedure. The amount of coupling reagent should be confirmed after optimized with experiment.

5. Wash (Solution A)

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 small molecules in the media. Ensure sufficient washing.

6. Wash (Solution C and Solution D)

Resuspend the blocked media with 5 times volume of C Solution. 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 small molecules 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 Solution E and drain the liquid. Store the media by immersing with Solution E.

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

8. Preparation of solution

Solution A: Purified water, adjust the pH to 4.5 with HCl. Prepare fresh solution before use.

Note: The specific pH depends on the stability of ligand, and the adjustment range is 4.5~6.5. A certain concentration (less than 50%) of organic solvent can be added if the adopted coupling reagent has poor solubility in aqueous solution.

Solution B: 0.5 M NaCl, adjust the pH to 4.5 with HCl. Store at room temperature.

Note: The specific pH depends on the stability of ligand, and the adjustment range is 4.5~6.5.

Solution C: 0.1M NaAc, 0.5 M NaCl, pH 4.0. Store at room temperature.

Note: C solution is used to wash out the small molecules which are defective tightness coupled. The same amount of organic solvent should be added into Solution C if the Solution A contains organic solvent.

Solution D: 0.1M Tris-HCl, 0.5M NaCl, pH 8.0. Store at room temperature.

Note: D solution is used to wash out the small molecules which are defective tightness coupled. The same amount of organic solvent should be added into Solution D if the Solution A contains organic solvent.

Solution E: 20% Ethanol. Store at room temperature.

Note: E solution is used for storage of media.

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 C.
   (3) Wash the media with 5~10 column volumes of purified water.
   (4) Wash the media with 5~10 column volumes of Solution D.
   (5) Wash the media with 5~10 column volumes of purified water.
   (6) Store the media after washed with 5~10 column volumes of Solution E.

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 0.2% 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 small molecules. 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 of 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.