

MMC Focurose HPL

Catalog No: E-CM-IE37

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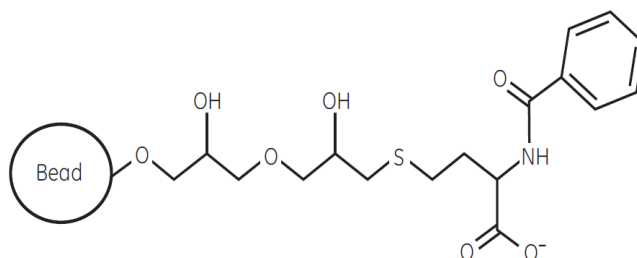
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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

MMC Focurose HPL is a multi-mode salt-tolerant biological separation media, suitable for group separation and moderate purification of all charged biomolecules such as proteins, peptides, nucleic acids, etc.

The ligand of MMC Focurose HPL is a multimodal ligand, which has many types of interactions with target molecules, mainly ionic interactions, followed by hydrogen bonding and hydrophobic interactions.



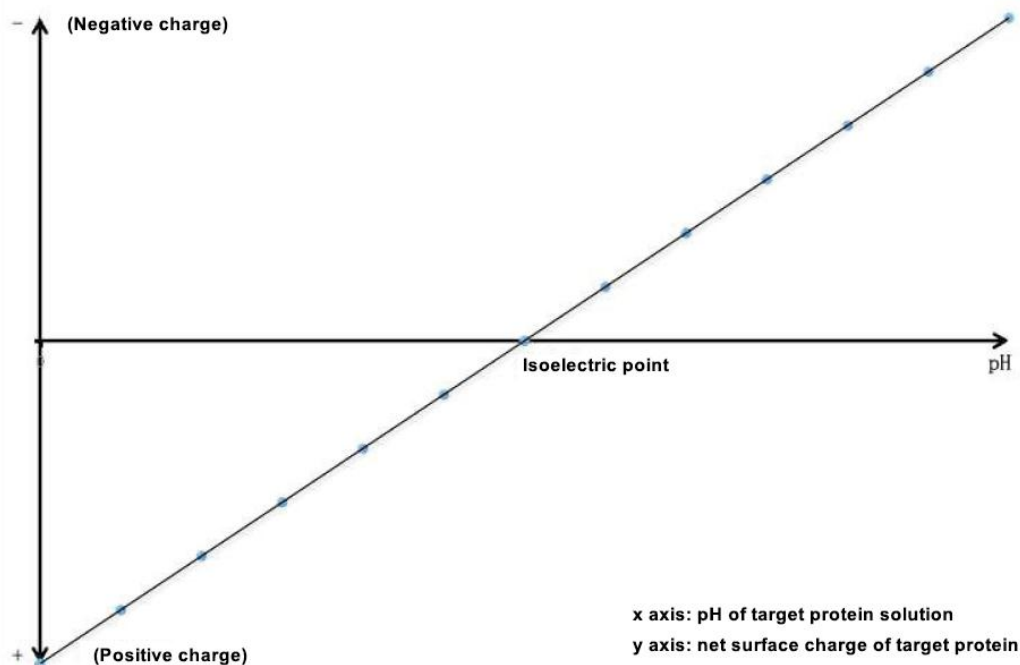
Advantages

1. Combining charged molecules under high conductivity conditions, the sample is no longer limited by the conductivity, and the sample can be ion exchanged without pre-treatment.
2. Large aperture, high capacity.
3. Compared with traditional ion exchange media, it has new selectivity.
4. High resolution.

Table 1: Performance index

Matrix	Highly rigid agarose
Particle size range	45-165 μm
Average particle size	75 μm
Media type	Weak cation exchange
Ionic capacity	90-120 $\mu\text{mol H}^+/\text{mL}$ (media)
pH stability	2-14 (short term), 3-12 (long term)
Chemical stability	All of the commonly used buffers, 1M NaOH, 8M urea, 6M guanidine hydrochloride, 70% Ethanol. Avoid using oxidants, cationic detergents, cationic buffers.
Maximum flow rate	300 cm/h
Pressure	≤ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~30 $^{\circ}\text{C}$

Selection of ion exchange chromatographic media



Instruction:

1. Choose the cation exchange medium when pH of target solution $<$ isoelectric point of target.
2. Choose the anion exchange medium when pH of target solution $>$ isoelectric point of target.
3. When choosing an ion exchange medium, the pH of all the applied solutions should be within the application pH range of ion exchange media.
4. When choosing an ion exchange medium, the pH, salt type, and salt concentration of all the applied solutions should be able to maintain the activity of target, so as to avoid acid/ alkali hydrolysis and precipitation of target.

Selection of buffer

Table 2: Cation exchange buffer

pH range	Salt	Concentration (mM)	Counterion	pKa(25°C)
2.6-3.6	Citric acid	20	Na ⁺	3.13
3.3-4.3	Lactic acid	50	Na ⁺	3.86
4.3-5.3	Acetic acid	50	Na ⁺ or Li ⁺	4.75
5.2-6.2	Methyl malonic acid	50	Na ⁺ or Li ⁺	5.76
5.6-6.6	MES	50	Na ⁺ or Li ⁺	6.27
6.7-7.7	Phosphate	50	Na ⁺	7.20
7.0-8.0	HEPES	50	Na ⁺ or Li ⁺	7.56
7.8-8.8	BICINE	50	Na ⁺	8.33

Instruction:

1. The type and concentration of buffer solutions must be selected strictly according to Table 2.
2. Wrong buffer (type and concentration) may interfere the separation effect, which mainly reflects on the effects of separation degree, loading of ion exchange medium, pH fluctuation in separation and purification process.
3. When choosing an ion exchange medium, the pH of all the applied solutions should be within the application pH range of ion exchange media.
4. All buffer reagents must use reagents that are analytically pure or of higher purity.
5. Solutions must be operated with filtration (particle size $\leq 45 \mu\text{m}$, 0.22 μm . particle size $\leq 165 \mu\text{m}$, 0.45 μm . particle size $\leq 300 \mu\text{m}$, 0.8 μm . Avoid of blocking of ion exchange medium) and degassing (affect the separation effect).

Preparation of sample

1. Titrate the sample with a suitable high-concentration buffer (refer to Table 2) to make the pH 0.5 to 2.0 lower than the isoelectric point of the target molecule.
2. Filtration of sample (particle size $\leq 45 \mu\text{m}$, 0.22 μm . particle size $\leq 165 \mu\text{m}$, 0.45 μm . particle size $\leq 300 \mu\text{m}$, 0.8 μm . Avoid of blocking of ion exchange medium).

Note: It is not recommended to directly adjust the pH of sample solution with strong acid/ strong alkali, which may lead to degradation and inactivation of target protein.

Selection of purification mode

According to the elution conditions, it can be divided into: Isometric elution (no need for precise chromatography equipment) and linear gradient elution (precise chromatography equipment is conducive to the improvement of resolution)

Note: Based on the type of ligand, it is recommended not to perform linear gradient elution only by changing the salt concentration.

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 10 CV of high ionic concentration eluent (20mM PB, 2M NaCl, pH7.5), then wash the media with 5 CV of purified water.

Note: This procedure is used to remove strong ion binding substances.

2. Wash the media with 10 CV of 1M NaOH and stand for 1~2 hour, then wash the media until the pH to neutral with purified water.

Note: This procedure is used to remove the precipitated proteins, lipids and denatured substances accumulated in 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).

Trouble shootings

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.
	The impurities or target is uncharged or charged with the same with the media.	Select the appropriate binding buffer.
	Improper detergents were added to the sample.	Check if there is improper detergent in the sample.
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.	The elution capacity of the eluent is not enough. Adjust the pH or increase the salt concentration of eluent.
	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 turns smooth and keep consistence with equilibrium liquid.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	Poor elution condition.	Optimize the elution condition.
	The target compound degrades.	Determine the stability of target

		compound.
	Bad loading effect of column resin.	Reload or re-purchase.
	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 was oxidized or 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.