Elabscience®

7th Edition, revised in April, 2017

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MMC Focurose 6FF (MMC-6FF)

Catalog No: E-CM-IE22

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.

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

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-6FF is a kind of multi-mode salt tolerant biological separation medium, which is suitable for group-separation and moderate purification of all types of charged biomolecules, such as proteins, peptides, nucleic acids, etc.

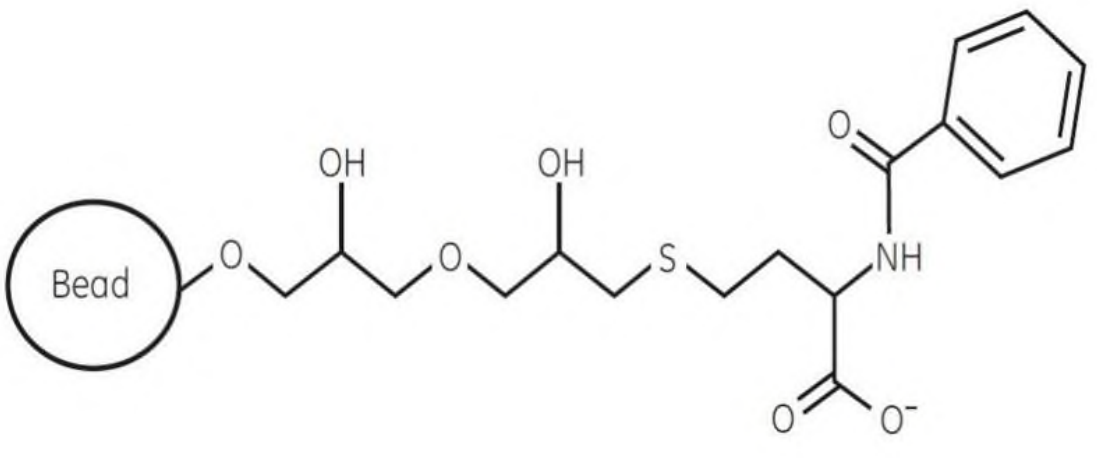


Figure 1: The ligand of MMC-6FF is a kind of multi-mode ligand, it has many types of interaction with target molecules, mainly include the ionic interaction, and followed by hydrogen binding and hydrophobic interaction, etc.

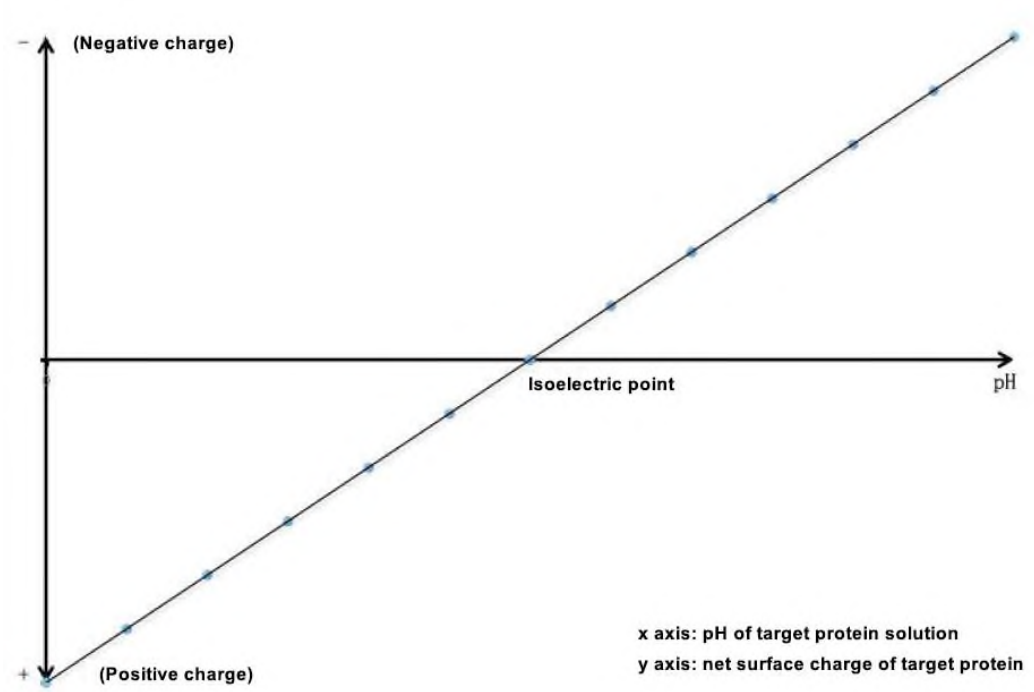
Advantages

1. Combine with charged molecules under high conductance conditions (refers to Figure.3). The sample is no longer restricted by electrical conductivity and can processed ion exchange without pretreatment.
2. High binding capacity. It has a higher processing capacity under flowthrough mode.
3. It has a new selectivity compared with the traditional ion exchange media.

Table 1: Performance index

Matrix	Highly cross-linked 6% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Media type	Weak cation exchanger
Ionic capacity	150-250 μmol H^+/mL (media)
Binding capacity	≥75 mg BSA/mL (media)
pH stability	2-14 (short term) 2-12 (working) 2-12 (long term)
Chemical stability	All of the commonly used buffers, 1M acetate, 1M NaOH, 70% Ethanol, 8M urea, 6M Guanidine hydrochloride. Avoid of oxidizing agent, cationic detergents and cationic buffer
Flow rate	300-600 cm/h (0.3MPa, XK16/40. Column bed height:30 cm)
Pressure	≤ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~30℃

Figure 2: 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℃)
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 pure or of higher purity.
5. Solutions must be operated with filtration (particle size ≤ 45 μm, 0.22 μm. particle size ≤ 165 μm, 0.45 μm. particle size ≤ 300 μm, 0.8 μm. Avoid of blocking of ion exchange medium) and degassing (affect the separation effect).

Preparation of sample

1. Titrate the sample with appropriate high concentration buffer (refers to Table. 2) to make its pH lower than the pI of target molecule 0.5 to 2.0 unit.
2. Filtration of sample (particle size ≤ 45 μm, 0.22 μm. particle size ≤ 165 μm, 0.45 μm. particle size ≤ 300 μm, 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 condition, it can be classified into **Isocratic elution** (No precise chromatography equipment is required) and **Linear gradient elution***(precise chromatography equipment is helpful to improve the resolution).

Note: Considering the ligand type, it is not recommended to operate the linear gradient elution only by changing the salt concentration.

Operation (MMC-6FF 1 mL or MMC-6FF 5 mL, here take the most commonly used methods for reference.)

1. Confirmation of pH in eluent

a. Preparation of equilibrium liquid

Choose the appropriate buffer according to Table 2, adjust pH to be the same as that of prepared sample solution.

b. Preparation of eluent

Prepare a series of buffers with different pH according to Table 2, adjust the pH to be 0.5 (eluent 1), 1.5 (eluent 2), and 2.5 (eluent 3) higher than the pI of target molecule, with a total of 3 types.

Note: The pH in the eluent can be further refined (e.g., 0.5, 1.0,1.5, 2, 2.5higher than the target molecules pI) if further optimization is required.

c. Preparation of sample

Prepare sample according to the sample preparation methods listed above.

d. Equilibration

Equilibrate the column with 5~10 CV of equilibrium liquid with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and set to zero until the baseline is smooth.

e. Sample application

Apply the sample with a flow rate of 0.5 mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL).

f. Wash

Wash with 5~10 CV of equilibrium liquid with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) until the baseline reaches zero.

g. Elution

Elute 5~10 CV of Eluent 1 with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and collect the eluted solution 1 until the baseline reaches zero. Elute 5~10 CV of Eluent 2 with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and collect the eluted solution 2 until the baseline reaches zero. Elute 5~10 CV of Eluent 3 with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and collect the eluted solution 3 until the baseline reaches zero.

h. Clean

Clean and regenerate the column according to the cleaning and regeneration method of step

Cleaning.

i. Analysis of result

The start material, flowthrough, eluted solution 1, eluted solution 2 and eluted solution 3 are performed with SDS-PAGE and activity detection. The pH in eluent when the target molecule appeared in the eluted solution is determined to be the elution pH.

2. Confirmation of salt concentration in eluent

a. Preparation of equilibrium liquid

Choose the appropriate buffer according to Table 2, adjust pH to be the same as that of prepared sample solution.

b. Preparation of eluent

Choose the appropriate buffer according to the pH confirmed in the above process (according to the Table 2), prepare a series of eluent with different salt concentration (NaCl) of 0.5M (Eluent 1), 1.0M (Eluent 2), 1.5M (Eluent 3), and adjust the pH to elution

Note: The salt concentration in the eluent can be further refined (e.g., 00.1M, 0.2M, 0.3M, 0.4M, 0.5M, 1.0M, 1.5M) if further optimization is required.

c. Preparation of sample

Prepare sample according to the sample preparation methods listed above.

d. Equilibration

Equilibrate the column with 5~10 CV of equilibrium liquid with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and set to zero until the baseline is smooth.

e. Sample application

Apply the sample with a flow rate of 0.5 mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL).

f. Wash

Wash with 5~10 CV of equilibrium liquid with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) until the baseline reaches zero.

g. Elution

Elute 5~10 CV of Eluent 1 with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and collect the eluted solution 1 until the baseline reaches zero. Elute 5~10 CV of Eluent 2 with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and collect the eluted solution 2 until the baseline reaches zero. Elute 5~10 CV of Eluent

3 with a flow rate of 0.5mL/min (MMC-6FF 1 mL) or 2.0 mL/min (MMC-6FF 5 mL) and collect the eluted solution 3 until the baseline reaches zero.

h. Clean

Clean and regenerate the column according to the cleaning and regeneration method of step **Cleaning.**

i. Analysis of result

The start material, flowthrough, eluted solution 1, eluted solution 2 and eluted solution 3 are performed with SDS-PAGE and activity detection. The salt concentration in eluent when the target molecule not appeared in the eluted solution is determined to be the elution salt concentration.

3. Confirmation of the optimum purification method

Validate the optimum purification process according to the conclusions of process 1 and 2.

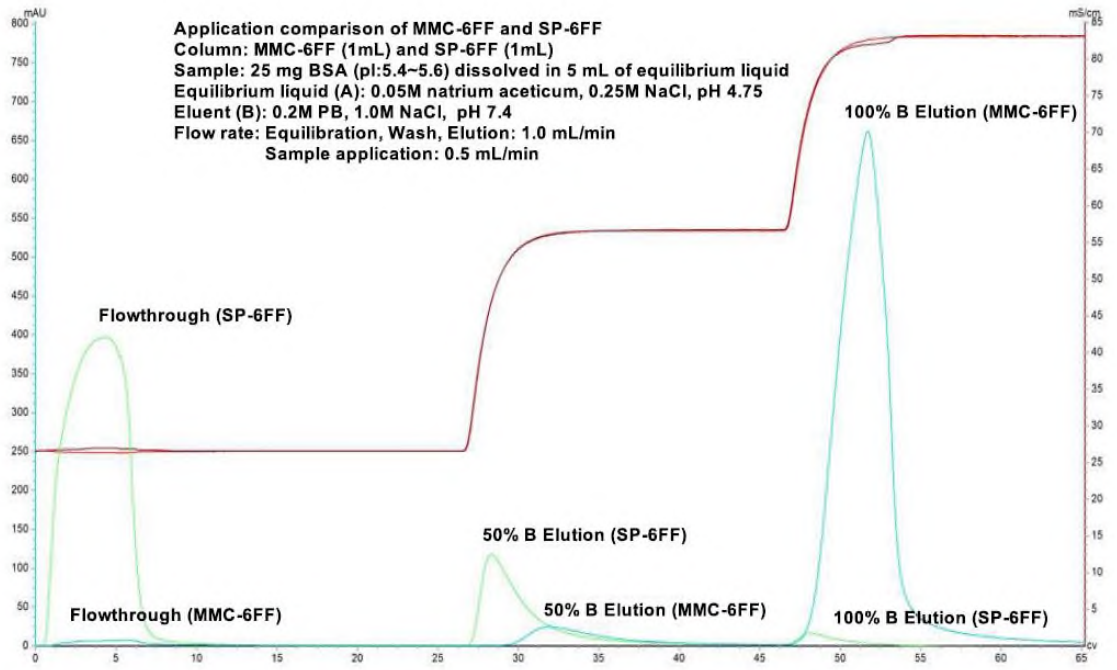
Cleaning

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 eluent with high ionic strength (20 mM PB, 2M NaCl, pH 7.5), then wash the media with 10 CV of purified water.
   Note: This procedure is used to remove the strong ionic binding substances.
2. Wash the media with 10 CV of 1M NaOH and let it stand for 1~2 hours, then wash the media with purified water to neutral.
   Note: This procedure is used to remove the precipitates, lipids and 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℃ (4~8℃ is preferred).

Comparison of protein binding between MMC-6FF and conventional cation exchange medium under high conductance



Conclusion: Under high salt condition, charged biomolecules are bound to MMC-6FF without the effect of electrical conductivity.

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.
	Poor binding condition.	Optimize the binding condition (pH and conductance).
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.