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

Focurose 4FF

Catalog No: E-CM-GF07

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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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

Focurose 4FF is suitable for component separation and medium purification of macromolecular substances (removal of small molecular impurities), such as virus particles, macromolecular proteins, recombinant hepatitis B surface antigen, polysaccharides and macromolecular complexes.

Advantages

1. High (physical and chemical) stability, high flow rate (component separation), high recovery rate (up to 95%).
2. The moderate elution conditions can completely preserve the biological activity and function of biological macromolecules.
3. Easy to be implemented.
4. Easy to maintain.

Table 1: Performance index

Matrix	Highly cross-linked 4% agarose
Particle size range	45-165 μm
Average particle size	≈90 μm
Exclusion limit (globulin)	60-20000kDa
pH stability	2-12 (long term) 2-14 (short term)
Chemical stability	2M NaOH, 70% Ethanol, 30% Isopropyl alcohol, 30% Acetonitrile, 1% SDS, 8M urea, 6M Guanidine hydrochloride
Flow rate	250-600 cm/h
High temperature and pressure	121℃ ×20 min, in water
Pressure	⩽0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~30℃

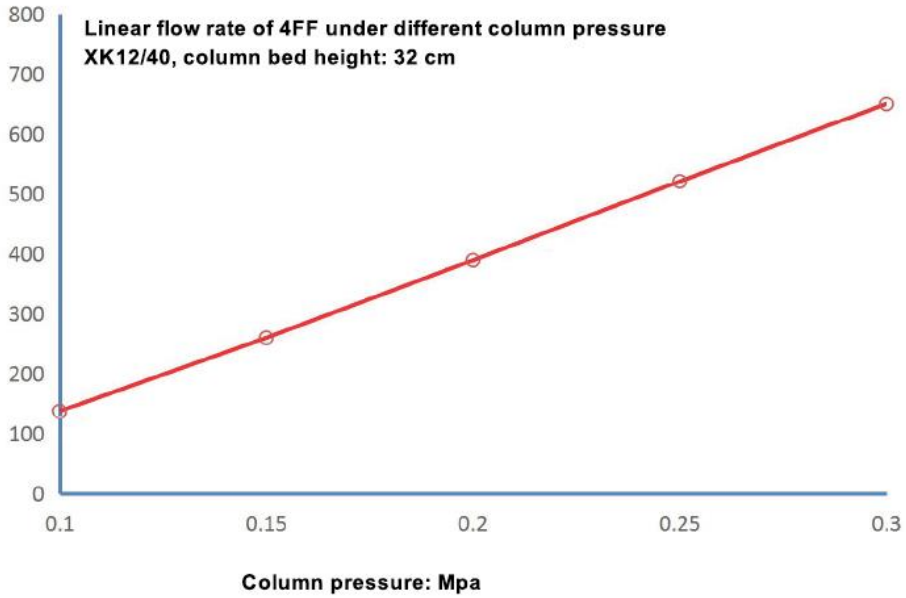
Preparation of buffer

Eluent: Prepared according to customer needs. It is recommended to add a certain concentration of salt (at least 0.025M) to the target solution to inhibit the ionic effect of the sample and media.

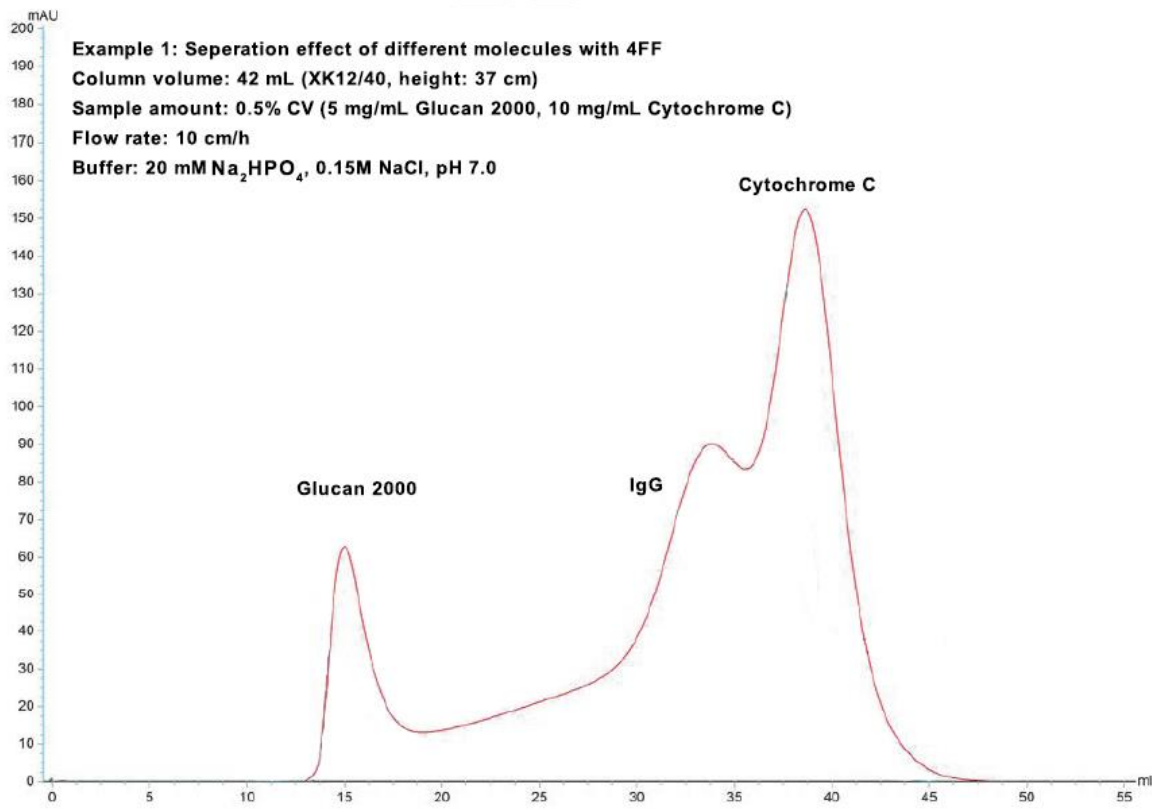
Preparation of sample

Filtration of sample (particle size ≤ 45 μm, 0.22 μm. particle size ≤ 165 μm, 0.45 μm. particle size ≤ 300 μm, 0.8 μm.

Figure 1: Linear flow rate of 4FF under different column pressure



Application



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 5-10 times. The exact washing frequency should be adjusted according to the cleanliness of the purified sample.

1. Wash the media with 2 CV of purified water with the flow rate of 1 mL/min.
2. Wash the media with 2 CV of 1M NaOH with the flow rate of 1 mL/min.
3. Wash the media with 2 CV of purified water with the flow rate of 1 mL/min.
4. Wash the media with 2 CV of 1M acetic acid with the flow rate of 1 mL/min.
5. Wash the media with 2 CV of purified water with the flow rate of 1 mL/min.
6. Wash the media with 2 CV of 70% ethanol with the flow rate of 1 mL/min.
7. Wash the media with 2 CV of purified water with the flow rate of 1 mL/min.
8. Store the media after washed with 2 CV of 20% ethanol with the flow rate of 1 mL/min.
   Note: Preserved buffer is 20% ethanol.

Sterilization

1. Wash the media with 2 CV of purified water with the flow rate of 1 mL/min.
2. Wash the media with 2 CV of 1M NaOH with the flow rate of 1 mL/min.
3. Wash the media with 2 CV of purified water with the flow rate of 1 mL/min.
4. Store the media after washed with 2 CV of 20% ethanol with the flow rate of 1 mL/min.
   Note: Preserved buffer is 20% ethanol.

Trouble shootings

Problem	Possible cause	Suggestion
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.
Low resolution	Inappropriate media.	Confirm whether the applied medium is appropriate.
	Poor column efficiency.	Determine the column efficiency.
	Overloading of sample volume.	Optimize the optimum sample amount.
	Flow rate is too fast.	Optimize the optimum flow rate.
	There is microbial grow in the media.	Change the medium.
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.

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