Elabscience®

9th Edition, revised in January, 2021

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

PS Focurose HPL

Catalog No: E-CM-AF30

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

PS Focurose HPL is a specific affinity resin, can be suitable for the separation and purification of some viruses, virus-like particles, and some specific antigens and proteins (see Table 2).

Advantages

1. Quick and easy (one-step purification).
2. High capacity.
3. Easy to be implemented.

Table 1: Performance index

Matrix	Highly rigid agarose
Particle size range	45-165 μm
Average particle size	90 μm
Lysozyme capacity	≥3 mg/mL (media)
pH stability	5-12
Flow rate	300 cm/h (16mm×300mm, 0.1MPa)
Pressure	≤ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~8℃

Table 2: Applicability of PS Focurose HPL

Viruses	Viral/Microbial Agents
Rabies	Herpes Simplex gA and gB Glycoprotein Subunits
Influenza	Hepatitis B Surface Antigen
Japanese Enchephalitis	Filamentous Hemagglutinin from B. pertussis
Feline Leukemia	Leucocytosis Promoting Factor Hemagglutinin
Feline Herpes	
Feline Calicivirus	
Respiratory Syncytial Virus	
Human Herpes Simplex	
Human Measles	
Human Parainfluenza	

Preparation of sample

1. The pH and component in sample must be consistent with the equilibrium liquid.
2. Filtration of sample (filter with 0.22μm when particle size ≤45μm, filter with 0.45μm when particle size ≤165μm, filter with 0.8μm when particle size ≤300μm).

Note: It is not recommended to directly adjust the pH of the sample solution with strong acids or bases, as the target compound may be degraded and inactivated.

Operation (take 1 mL column and 5 mL column for example)

1. Wash (water)

Wash the media with 5~10 CV (column volume) of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This operation is used to remove the 20% ethanol in media.

2. Equilibration

Balance the media with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) until the baseline turns stable and then set to zero.

Note: This procedure is used to balance the media. Make sure that the pH and component in media are in accordance with sample.

3. Sample application

Apply the sample with flow rate of 0.2 mL/min (1 mL) or 1.0 mL/min (5 mL) after centrifugation and filtration (0.45 μm). Wash with the equilibrium liquid until the baseline tends to zero.

Note: The binding capacity of media varies with the sample. Low flow rates often increase the binding efficiency of samples.

4. Elution

Elute with 5~10 CV of eluent with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Note: Low flow rate may increase the concentration of target protein in the eluted solution.

5. Wash with water

Wash the media with 5~10 CV of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This procedure is used to remove the eluent in media.

6. Storage

Wash the media with 5~10 CV of 20% ethanol with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and the store the media.

Note: 20% ethanol can prevent the growth of microorganism.

7. **Preparation of buffer**

   Equilibrium liquid: 0.01M PB, adjust the pH to 7.2±0.2. Store the prepared equilibrium liquid at 4-8℃.

   Note: 0.1M NaCl in equilibrium liquid is used to inhibit the adsorption of impurities.

   Eluent: 0.01M PB, 1M NaCl, adjust the pH to 7.2. Store the prepared eluent at room temperature.

   Note: The concentration of NaCl can be increased to 2M to improve elution effect.

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 10-20 times. The exact washing frequency should be adjusted according to the cleanliness of the purified sample.

1. Wash the media with 10 CV of 3M NaCl with flow rate of 50 cm/h.
2. Wash the media with 10 CV of 0.11M NaOH with flow rate of 50 cm/h.
3. Wash the media with 10 CV of 3M NaCl with flow rate of 50 cm/h.
4. Wash the media with 5 CV of purified water with flow rate of 100 cm/h until UV and conductivity are stable.
5. Store the media at 4-8℃ after washed with 5 CV of 20% ethanol with flow rate of 100 cm/h.
   Note: 20% ethanol can prevent the growth of microorganism.

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.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	The target is not charged or has the same charge as the media	Screen the appropriate binding buffer
	Incorrect salt concentration and pH in the sample or equilibrium liquid.	Check the conductivity and pH in the sample and equilibrium liquid.
	Wrong buffer solution was used.	Refer to the buffer selection table.
	Unsuitable detergent was added to the sample	Check if there is an inappropriate 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.
	Improper elution conditions	Increase the salt concentration and adjust the pH of the 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.	Check the solubility and stability of target compound in the elution (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 is stable and consistent with equilibrium liquid.

	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	Poor elution conditions	Optimize elution conditions
	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 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 eluent 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.