Elabscience®

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IgM Focurose 6HP

Catalog No: E-CM-AF28

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

IgM Focurose 6HP can be used for separation and purification with the interactions (high salt enhancement, low salt weakening) between electron donor and electron acceptor, and it is suitable for separation and purification of monoclonal antibodies from hybridoma cell culture supernatants or human IgM.

Advantages

1. Quick and easy (one-step purification).
2. Fast flow rate, high yields and easy to be implemented.

Performance index

Matrix	Highly cross-linked with 6% agarose
Particle size range	25-45 μm
Average particle size	37 μm
Binding capacity	About 5 mg (human IgM)/mL (media)
pH stability*	3-11 (long-term) 2-13 (short term)
Chemical stability*	All of the common buffers, 30% isopropanol, 70% ethanol, 1M acetic acid, 0.1M NaOH
Flow rate	300 cm/h
Pressure	⩽ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~8℃

Note: The binding capacity of the media will vary depending on the species and subtype of the sample.

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

Slowly add ammonium sulfate powder or saturated ammonium sulfate solution to the sample until the ammonium sulfate concentration is consistent with the equilibrium liquid. 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: For IgM monoclonal antibodies with low binding capacity or no binding, the binding capacity can be increased by increasing the concentration of ammonium sulfate to 1 M. However, as the concentration of ammonium sulfate increases, the binding capacity of IgG will increase, so the sample should be added less serum or not added.

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: 0.02M PB, 0.8M $(NH_4)_2SO_4$, adjust the pH to 7.5. Store the prepared equilibrium liquid at 4-8℃.

Note: 0.5M potassium sulfate can replace ammonium sulfate, and its purification effect is not significantly different from that of 0.8M ammonium sulfate.

Eluent: 0.02M PB, adjust the pH to 7.5. Store the prepared eluent at 4-8℃.

Note: Prepare the fresh eluent before use and store at 4℃. Because the eluent is highly susceptible to microbial growth.

Washing buffer: 0.02M PB, 30% isopropanol, adjust the pH to 7.5. Store the prepared solution at room temperature.

Note: The concentration of isopropanol in the washing buffer should not be too high, otherwise it will lead to the precipitation of IgM.

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 5~10 CV of purified water.
   Note: This procedure is used to remove 20% ethanol in the media.
2. Wash the media with 10~20 CV of washing buffer, then wash the media with 5~10 CV of purified water immediately.
   Note: This procedure is used to remove strongly binding substances or residue IgM.
3. Wash the media with 10~20 CV purified water.
   Note: This procedure is used to remove washing buffer.
4. Store the media at 4-8℃ after washed with 5~10 CV of 20% ethanol.
   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.
	Weak binding ability between target compound and media	Increase the concentration of $(NH_4)_2SO_4$ in equilibrium liquid.
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.
	Insufficient elution time.	Decrease the flow rate and prolong the retention time of eluent.
	Insufficient elution volume.	Increase the elution volume.
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.
	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.
	There is microbial grow in the media.	Filter and degas all the reagents. Samples must be centrifuged or filtered before loading.