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Phenyl Focurose 6FF (LS) (Phenyl-6FF (LS))

Catalog No: E-CM-HI02

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)
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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

Phenyl-6FF (LS) can interact with some hydrophobic protein or antibody under high ionic strength conditions (high ionic strength may increase the interaction between ligand and hydrophobic groups), thus to achieve the purpose of separation and purification. Phenyl-6FF (LS) is mainly used for capture of initial samples and moderate purification of samples.

Advantages

1. Rapid, easy to use (one-step purification).
2. Compared with reversed-phase chromatography, the ligand concentration in the hydrophobic interaction chromatography media is low and the elution conditions are mild, which helps to maintain the biological activity of biomolecules.
3. Wide application. Phenyl-6BB can be applied in preliminary capture and moderate purification, and it can be repeatedly used in combination ion exchange chromatography media.

Table 1: Performance index

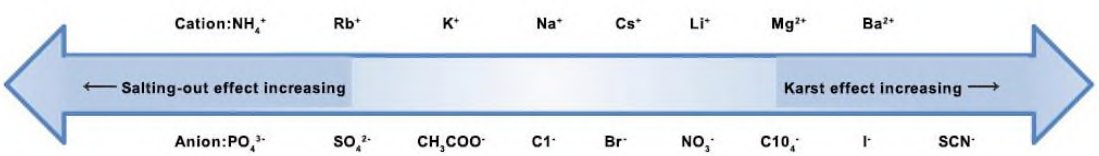
Ligand	Phenyl
Matrix	Highly cross-linked 6% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Ligand density	15-20 μmol/mL
Binding capacity	15 mg (IgG)/mL (media)
pH stability	2-14 (short-term) 3-13 (long-term)
Chemical stability	All of the commonly used buffers, 8M urea, 6M guanidine hydrochloride
Flow rate	450 cm/h
Storage buffer	20% Ethanol
Storage temperature	4~30℃ （4~8℃ is preferred)

Table 2: Factors affecting hydrophobic chromatography

Influence	Function mechanism	Suggestion
Ligand structure	The binding ability between different ligands and proteins is different.	Pre-experiment is recommended to screen suitable media, which can be referred to in Figure 1.
Ligand density	The higher the concentration of ligand, the stronger the binding ability will be.	Pre-experiment is recommended to screen the optimum ligand density.
Sample properties	The hydrophobicity of protein depends on the distribution of hydrophobic groups on its surface.	/
Salt concentration	The higher the salt concentration, the stronger the binding between the ligand and the protein will be, but the excessive high concentration of salt may result in protein precipitation.	Check the solubility and stability of protein under different salt concentrations.
Salt type	Different kinds of salts can result in different binding effects.	$(NH_4)_2SO_4$ and NaCl are preferred, other selections can refer to Figure 2.
Temperature	The higher the temperature, the stronger the protein hydrophobic will be.	Temperature must be maintained to be the same. Room temperature is recommended.
pH	Excessive high or low pH may affect the solubility and stability of protein, and pH can affect the binding effect.	The recommended pH range is 5.0-8.5 on the premise of ensuring the solubility and stability of protein.

Figure 1: Hydrophobic properties comparison of ligands with different concentrations

Figure 2: Salt dissolution and salting out effect of different ions



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 (HT 1 mL) or 2.0 mL/min (HT 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 (HT 1 mL) or 2.0 mL/min (HT 5 mL) until the baseline turns stable and then set to zero.

Note: This procedure is used to balance the media. Ensure 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: Make sure that the ionic strength and pH of sample solution must be the same with the equilibrium liquid.

4. **Elution (Choose the elution mode according to the equipment condition)**

Linear gradient elution (with chromatographic system): Elute with 20 CV of 0%-100% eluent at a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Stepwise elution (with peristaltic pump): Stepwise elute with 20 CV of 0%-100% eluent at a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Note: Linear gradient elution is strongly recommended. A series of eluent* with different salt concentration should be prepared for stepwise elution.

*Eluent Ⅰ: 0.05M PB, 1.50M $(NH_4)_2SO_4$, pH 7.0.
Eluent Ⅱ: 0.05M PB, 1.25M $(NH_4)_2SO_4$, pH 7.0.
Eluent Ⅲ: 0.05M PB, 1.00M $(NH_4)_2SO_4$， pH 7.0.
Eluent Ⅳ: 0.05M PB, 0.75M $(NH_4)_2SO_4$， pH 7.0.
Eluent Ⅴ: 0.05M PB, 0.50M $(NH_4)_2SO_4$， pH 7.0.
Eluent Ⅵ: 0.05M PB, 0.25M $(NH_4)_2SO_4$， pH 7.0.
Eluent Ⅶ: 0.05M PB, pH 7.0.

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. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is better).

7. Preparation of buffer

Equilibrium liquid: 0.05M PB, 1.7M $(NH_4)_2SO_4$, adjust the pH to 7.0. Store the prepared equilibrium liquid at room temperature.

Note: Some proteins may precipitate under high salt concentration. It is recommended to determine the protein solubility and stability under different salt concentration. pH should be ≤8.0 when using $(NH_4)_2SO_4$.

Eluent: 0.05M PB, adjust the pH to 7.0. Store the prepared eluent at room temperature.

Storage buffer: 20% ethanol, store at room temperature.

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 5~10 CV of purified water.
   Note: This procedure is used to remove the 20% ethanol in media.
2. Wash the media with 5~10 CV of 1M NaOH and stand for 1 hour, then wash the media to neutral with purified water.
   Note: This procedure is used to remove the precipitates or denatured substances accumulated in the media.
3. Wash the media with 5~10 CV of 70% ethanol or 30% isopropyl alcohol and stand for 1 hour, then wash the media with purified water immediately.
   Note: This procedure is used to remove the strong hydrophobic binding substances.
4. 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).

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 salt concentration of equilibrium liquid is low or the hydrophobicity of target is weak.	Increase the salt concentration of equilibrium liquid, or change the equilibrium liquid, or choose another type of media with stronger hydrophobicity.
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.
	The target and media combined too strong.	Decrease the salt concentration of equilibrium liquid, or change the salt type, or choose another type of media with weaker hydrophobicity, or add additives into the eluent (a small amount of detergent or low concentration organic reagent).
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 smooth and keep consistence with equilibrium liquid.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	Poor elution condition, fast elution speed and abrupt elution gradient.	Adjust the elution condition.
	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.
	Wrong type of media.	Select the suitable hydrophobic media.
	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 oxidized or dropped off due to excessive use.	Wash the media timely or reload 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.