Elabscience®

9th Edition, revised in January, 2021

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ANX Focurose 4FF

Catalog No: E-CM-IE18

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

ANX Focurose 4FF is suitable for separation and purification of all types of charged biomolecules, such as proteins, peptides, nucleic acids, etc.

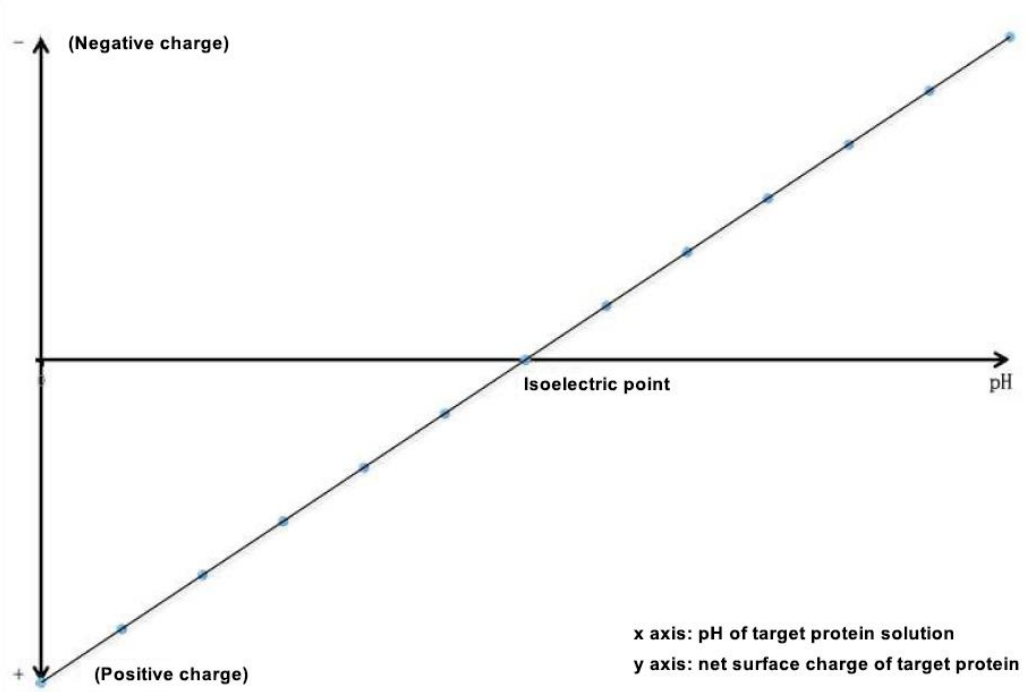
Advantages

1. Fast flow rate, increase production capacity.
2. Medium granule, good resolution.
3. Good compatibility, suitable for initial capture or intermediate purification of biomolecules of vario us scales.
4. The purification process has high flexibility and can be used in combination with hydrophobic chro matography.

Table 1: Performance index

Ligand	ANX Focurose 4FF
Matrix	Highly cross-linked 4% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Media type	Weak anion exchanger
Charged group	$-N^+ (C_2H_5)_2 H$
Ionic capacity	130-170 μmol Cl^-/mL (media)
pH stability	2-14 (short term) 3-10 (long term)
Chemical stability	All of the commonly used buffers, 1M NaOH, 70% Ethanol, 8M urea, 6M Guanidine hydrochloride. Avoid of oxidizing agent, anionic detergent and anionic buffer
Flow rate	250 cm/h
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: Anion exchange buffer

pH range	Salt	Concentration (mM)	Counterion	pKa(25℃)
4.3-5.3	N-Methylpiperazine	20	Cl^-	4.75
4.8-5.8	Piperazine	20	Cl^- or $HCOO^-$	5.33
6.0-7.0	Bis-Tris	20	Cl^-	6.48
6.2-7.2	Bis-Tris propane	20	Cl^-	6.65
7.3-8.3	Triethanolamine	20	Cl^- or $CH3COO^-$	7.76
7.6-8.6	Tris	20	Cl^-	8.07
8.0-9.0	N-Methyldiethanolamine	20	SO_4^{2-}	8.52
8.4-9.4	Propane 1,3-Diamino	20	Cl^-	8.88
8.6-9.6	Bis-Tris propane	20	Cl^-	9.10
9.0-10.0	Ethanolamine	20	Cl^-	9.50
9.2-10.2	Piperazine	20	Cl^-	9.73
10.0-11.0	Propane 1,3-Diamino	20	Cl^-	10.55
10.6-11.6	Piperidine	20	Cl^-	11.12

Table 3: 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 and Table 3.
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 analytically pure or of higher purity.

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. The pH and salt components of sample must be the consistent with the equilibrium liquid, which can be performed the buffer exchange by dilution or dialysis with equilibrium liquid, ultrafiltration and gel filtration (G25).
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

1. Wash the media with 5-10 CV (column volume) of purified water with a flow rate of 150 cm/h.
2. Equilibrate the media with 5-10 CV of equilibrium liquid with a flow rate of 150 cm/h until UV, pH, and conductance turns stable.
3. Apply the sample to the column.
4. Wash the media with 5-10 CV of equilibrium liquid until no material flows through.
5. Linear gradient elution (preferred): Linear gradient elution 10-20 CV (0%-50% eluent).
   Isometric elution: elution is performed by gradually increasing the salt concentration in the equilibrium liquid, elute with 5 CV of each salt concentration eluent.
6. Wash the media with 5 CV of 100% eluent.
   Note: Eluent = equilibrium liquid + 1M NaCl, Other components remain unchanged.

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 5 CV of 2M NaCl with the flow rate of 50 cm/h for 1-2 h.
2. Wash the media with 5 CV of 1M NaOH with the flow rate of 50 cm/h for 1-2 h.
3. Wash the media with 5 CV of 2M NaCl with the flow rate of 50 cm/h for 1-2 h.
4. Wash the media with 5 CV of purified water with the flow rate of 50 cm/h until UV and conductance turns stable.
5. Store the media after washed with 5 CV of preserved buffer with the flow rate of 50 cm/h.
   Note: Preserved buffer is 20% ethanol or 0.1 M NaOH.

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 salt concentration and pH in sample solution or equilibrium liquid.	Check the pH and conductance of sample solution or equilibrium liquid.
	Improper buffer.	Refer to the buffer selection table.
	Improper detergents were added to the sample.	Check if there is improper 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.
	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.