Elabscience®

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Q Focurose 6HPR (Q-HPR)

Catalog No: E-CM-IE11

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

Q-HPR is suitable for group-separation, moderate purification and fine purification of all types of charged biomolecules, such as proteins, peptides, nucleic acids, etc.

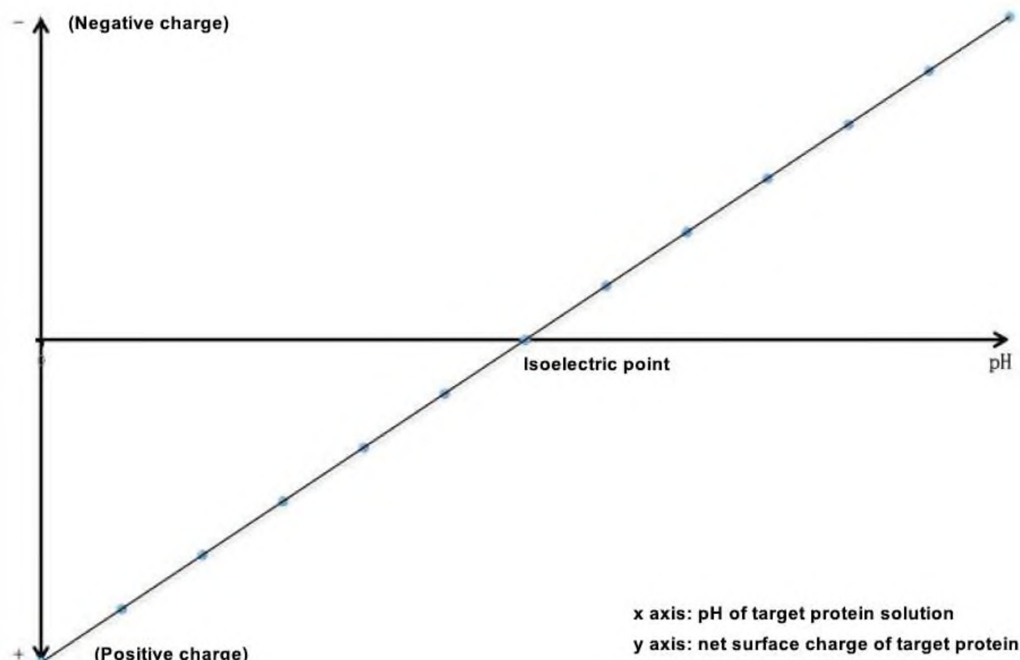
Advantages

1. Quick, easy, convenient.
2. Wide application range. It is suitable for component separation or fine purification of all types of charged biomolecules.
3. The purification process is highly flexible, and the purity of the sample can be improved by screening the purification conditions in earlier stage.
4. High pressure resistance, high resolution, high binding capacity.

Table 1: Performance index

Ligand	Quaternary amine
Matrix	Highly cross-linked agarose and cellulose
Particle size range	25-45 μm
Average particle size	37 μm
Media type	Strong anion exchanger
Charged group	$-N^{+} (CH_3)_3$
Ionic capacity	180-250 μmol Cl^{-}/mL (media)
Binding capacity	120 mg BSA/mL (media)
pH stability	2-14 (short term) 2-12 (working) 2-12 (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	300-400 cm/h (0.4MPa, XK16/40. Column bed height:30 cm)
Pressure	≤0.5 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 CH_3COO^-	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

Instruction:

1. The type and concentration of buffer solutions must be selected strictly according to Table 2.
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 pure or of higher purity.
5. 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. According to the elution method, it can be classified into **Salt concentration elution** (NaCl is commonly used) and **pH elution**.
   Note: It is recommended to choose the **Salt concentration elution** prior when choosing the purification method, because **pH elution** is difficult to achieve linear gradient.
2. According to the elution condition, it can be classified into **Isocratic elution** (No precise chromatography equipment is required) and **Linear gradient elution** (precise chromatography equipment is helpful to improve the resolution).
   Note: A more accurate linear gradient elution is recommended when choosing the purification method.

Operation

1. Screening of the optimal combination pH

a. Preparation of equilibrium liquid

Prepare a series of buffers with different pH according to Table 2, adjust the pH to be 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, with a total of 12 types.

b. Preparation of eluent

Prepare a series of buffers with different pH according to Table 2, adjust the pH to be 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 by adding 1M NaCl, with a total of 12 types.

c. Preparation of sample

Prepare sample (start material) with a series of different pH (with a total of 12 types) according to the sample preparation methods introduced above and store at 4℃.

d. Preparation of medium

Take the Q-HPR settling medium cleaned with equal amounts of purified water into 12 containers (marked 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5), 1.0 g for each container.

e. Equilibration

Take 20 mL of equilibrium liquid into the corresponding container, then resuspend the medium for 10 minutes. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out).

f. Sample application

Add V mL of sample into the corresponding container and mix for 1~2 hours at room temperature. After stand for 10 minutes, discard the supernatant (gently tilt the container, pour out the supernatant (flowthrough solution) as much as possible and ensure that the medium is not poured out) and store at 4℃.

Note: V is recommended to be 10~20 (both too large and too small sample amount can affect the binding). Make sure that the total amount of all the substances (targets and impurities) in the sample does not exceed the carrying capacity of the medium, or the result may be affected by overloading.

g. Wash

Take 10 mL of different equilibrium liquid into the corresponding container, mix for 10 minutes and then stand for 10 minutes at room temperature. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out). Repeat this step 2 times.

h. Elution

Take V/2 mL of different eluent into the corresponding container, mix for 10 minutes and then stand for 10 minutes at room temperature. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out). Dilute with corresponding equilibrium liquid (diluent) for 1 time and store at 4℃.

i. Clean

Mix all the used medium, then clean and regenerate the medium according to the cleaning and regeneration method of step **Cleaning.**

j. Analysis of result

The start material, flowthrough, eluted solution of different pH are performed with SDS-PAGE and activity detection. With the premise of ensuring activity, the minimum pH of the complete binding of sample is determined to be the optimal combination pH.

2. Screening of the optimal combination salt concentration

a. Preparation of equilibrium liquid

Prepare a series of buffers with different NaCl concentration (0.05 M, 0.10 M, 0.15 M, 0.2 M, 0.25 M, 0.30 M, 0.35 M, 0.40 M, 0.45 M, 0.5M), with a total of 10 types. Adjust the pH to be the optimal combination pH.

b. Preparation of eluent

Prepare the optimal buffer, add 1M NaCl. Adjust the pH to be the optimal combination pH.

c. Preparation of sample

Prepare sample (start material) with a series of different pH (with a total of 10 types) according to the sample preparation methods introduced above.

d. Preparation of medium

Take the Q-HPR settling medium cleaned with equal amounts of purified water into 10 containers (marked 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5), 1.0 g for each container.

e. Equilibration

Take 20 mL of equilibrium liquid into the corresponding container, then resuspend the medium for 10 minutes. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out).

f. Sample application

Add V mL of sample into the corresponding container and mix for 1~2 hours at room temperature. After stand for 10 minutes, discard the supernatant (gently tilt the container, pour out the supernatant (flowthrough solution) as much as possible and ensure that the medium is not poured out) and store at 4℃.

Note: V is recommended to be 10~20 (both too large and too small sample amount can affect the binding). Make sure that the total amount of all the substances (targets and impurities) in the sample does not exceed the carrying capacity of the medium, or the result may be affected by overloading.

g. Wash

Take 10 mL of different equilibrium liquid into the corresponding container, mix for 10 minutes and then stand for 10 minutes at room temperature. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out). Repeat this step 2 times.

h. Elution

Take V/2 mL of different eluent into the corresponding container, mix for 10 minutes and then stand for 10 minutes at room temperature. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out). Dilute with corresponding equilibrium liquid (diluent) for 1 time and store at 4℃.

i. Clean

Mix all the used medium, then clean and regenerate the medium according to the cleaning and regeneration method of step **Cleaning.**

j. Analysis of result

The start material, flowthrough, eluted solution of different pH are performed with SDS-PAGE and activity detection. With the premise of ensuring activity, the highest salt concentration solution when the target protein completely combined is determined to be the optimal binding solution of the target protein, and the minimum salt concentration when the target protein

complete not-combined is determined to be the highest salt concentration during elution.

3. Screening of the optimal eluent

a. Preparation of equilibrium liquid

Prepare the optimal equilibrium liquid according to concludes of Step 1 and 2 (including pH and salt concentration).

b. Preparation of eluent

Prepare a series of eluents with different salt concentration between the equilibrium liquid according to salt concentration (e.g., 0.1M) and the highest elution salt concentration (e.g., 0.3M) according to concludes of Step 1 and 2 (salt concentrations: 0.12M, 0.14M, 0.l6M, 0.18M, 0.20M, 0.22M, 0.24M, 0.26M, 0.28M, 0.30M, with a total of 10 types).

c. Preparation of sample

Prepare sample (start material) with a series of different pH (with a total of 10 types) according to the sample preparation methods listed above.

d. Preparation of medium

Take the Q-HPR settling medium cleaned with equal amounts of purified water into 9 containers (marked 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28, 0.30), 1.0 g for each container.

e. Equilibration

Take 20 mL of equilibrium liquid into the corresponding container, then resuspend the medium for 10 minutes. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out).

f. Sample application

Add V mL of sample into the corresponding container and mix for 1~2 hours at room temperature. After stand for 10 minutes, discard the supernatant (gently tilt the container, pour out the supernatant (flowthrough solution) as much as possible and ensure that the medium is not poured out) and store at 4℃.

Note: V is recommended to be 10~20 (both too large and too small sample amount can affect the binding). Make sure that the total amount of all the substances (targets and impurities) in the sample does not exceed the carrying capacity of the medium, or the result may be affected by overloading.

g. Wash

Take 10 mL of different equilibrium liquid into the corresponding container, mix for 10 minutes and then stand for 10 minutes at room temperature. Discard the supernatant (gently tilt

the container, pour out the supernatant as much as possible and ensure that the medium is not poured out). Repeat this step 2 times.

h. Elution

Take V/2 mL of different eluent into the corresponding container, mix for 10 minutes and then stand for 10 minutes at room temperature. Discard the supernatant (gently tilt the container, pour out the supernatant as much as possible and ensure that the medium is not poured out). Dilute with corresponding equilibrium liquid (diluent) for 1 time and store at 4℃.

i. Clean

Mix all the used medium, then clean and regenerate the medium according to the cleaning and regeneration method of step **Cleaning.**

j. Analysis of result

The start material, flowthrough, eluted solution of different pH are performed with SDS-PAGE and activity detection. The eluent with complete elution and the highest purity of target protein is determined to be the optimal eluent.

4. Confirmation of the optimal purification process

Validate the optimal purification process according to the conclusions of process 1, 2, 3.

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 10 CV of 2M NaCl, then wash the media with 10 CV of purified water.
   Note: This procedure is used to remove the strong ionic binding substances.
2. Wash the media with 10 CV of 1M NaOH and let it stand for 1~2 hours, then wash the media with purified water to neutral.
   Note: This procedure is used to remove the precipitates, lipids and denatured substances accumulated in the media.
3. 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 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.

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