Elabscience®

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Focudex G-75 Medium (G75 medium)

Catalog No: E-CM-GF10

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

G75 medium is a kind of gel filtration chromatographic medium (also known as size exclusion chromatography), it is suitable for desalination of biomolecules, buffer exchange, group separation and fine purification.

Advantages

1. Classical glucan medium with good selectivity.
2. Rapid desalination of biological macromolecules (>80kDa) and buffer replacement (one-step).
3. Fast flow rate. It can be used for group separation and removal of impurities (with the exclusion limit of 80 kDa as the cut-off point).

Performance index

Matrix	Cross-linked dextran
Particle size range (dry powder)	45-165 μm
Swelling ratio	12-15 mL/g
Particle size range (wet gel)	80-300 μm
Separation range	3-80kDa (Globular proteins) /1-50kDa (Dextran)
Exclusion limit	80kDa
pH stability	2-10 (long term) 2-10 (working) 2-13 (short term)
Chemical stability	All commonly used buffers. 8M Urea, 6M Guanidine hydrochloride, all ionic or nonionic detergents, ≤25% Methanol/ Ethanol/ Propyl alcohol (V/V). Avoid of extreme pH (<2 or >13) and oxidant.
Flow rate	≥40 cm/h (Inter diameter: 1.6 cm, Column bed height: 30 cm)
Pressure	≤0.3 MPa
Autoclaving	120℃×30min(wet gel, pH 7.0)
Storage buffer	20% Ethanol (for swelling media)
Storage temperature	4~30℃

Selection of G75

1. Selection of medium grade

(1) Rapid desalination, buffer replacement, group separation, and impurity removal: G75 medium, large particle diameter and high flow rate.

(2) Separation and purification of small molecular proteins: G75 superfine, slow flow rate and high resolution.

2. Selection of chromatographic column:

(1) Rapid desalination, buffer replacement, group separation, and impurity removal (the sample amount can reach 30% CV): Coarse chromatography column (low column height, e.g., XK 16/60), high flow rate and short cycle.

(2) Separation and purification of small molecular proteins (the sample amount is 0.5%~4% CV): Slender chromatography column (high column height, e.g., XK 16/60), high column efficiency and high resolution.

Operation

1. Swelling

Take a certain amount of dry powder and add an excess of purified water (Buffer: Dry powder ≥20 mL: 1 g). Swell the medium for 24 hours at room temperature (25℃) or for 3 hours in boiling water.

Note: During the swelling process, soft mixing can be operated and high speed or vigorous agitation (e.g., high speed magnetic agitation, etc.) should be avoided.

2. Wash

After the medium is settled naturally, carefully pour out the supernatant (including a small amount of dry powder and some small floating medium without swelling) and then add 3-5 times the purified water for suspension.

Repeat this step 5 times.

Note: This procedure is used to clean the trace organic reagents and alkaline substances in the medium.

3. Resuspend

Add a certain amount of buffer (Buffer: Medium =1 mL: 3 mL) into the clean medium. Gently mix with glass rod or other mixing device for 3-5 minutes.

4. Degassing

Degas the suspended medium with a vacuum pump or ultrasonic cleaning device.

Note: Degassing is an essential step for efficient column loading.

5. Column packing

(1) After gently mix the degassed medium with glass rod or other mixing device, add the medium into the chromatographic column quickly and continuously (draining with glass rods to avoid the introduction of bubbles).

(2) Press the column with a constant flow rate (not exceeding the maximum operating pressure of the medium) or maximum operating pressure of 95% to ensure continuous column of 2-3CV

(column volume) and no change in column height occurring in the last half an hour.

(3) Press the lower end of the transition joint gently (not to introduce bubbles) to the 3mm below the column bed surface.

(4) Equilibrate the chromatographic column (1 CV) with a flow rate of ≤75% column pressure. The column height may not change during the whole process, otherwise it must be reloaded.

6. Application

(1) For rapid desalination, buffer replacement, group separation and impurity removal, it is recommended that the initial sample size should be 20% CV.

(2) For the separation and purification of small proteins, it is suggested that the initial sample size should be 0.5% CV.

(3) When the separation effect has been met, the amount of sample can be raised gradually.

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. Clean in place

(1) Wash the media with 2~3 CV 0.2M NaOH, then wash the media to neutral with purified water.

(2) Store the media after washed with 1~2 CV of 20% ethanol.

2. Clean the medium separately

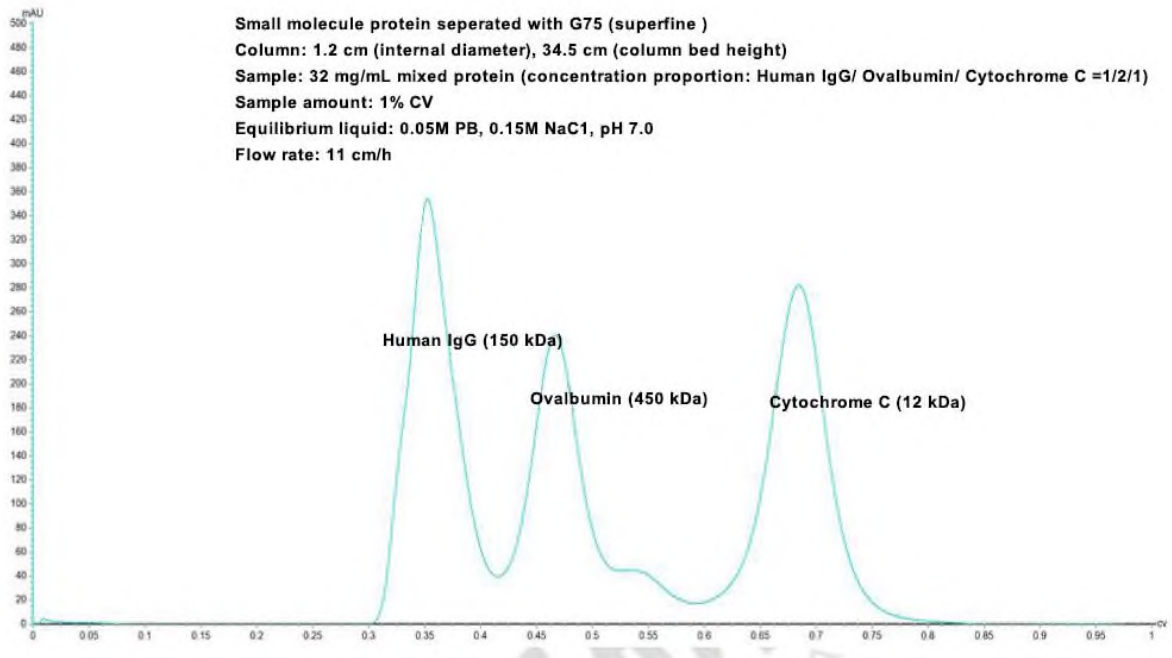
(1) Immerse the medium with 1~2 medium volume of low concentrations of ionic or nonionic detergents (e.g., 0.5%Triton X-100) for 0.5 hour, gently pour off the supernatant after settling. Resuspend the medium with 2 CV of purified water, gently pour off the supernatant after settling. Repeat washing with purified water for 3 times.

(2) Immerse the medium with 1~2 medium volume of 0.2M NaOH for 1 hour, gently pour off the supernatant after settling. Resuspend the medium with 2 CV of purified water, gently pour off the supernatant after settling. Repeat washing with purified water for 3 times.

(3) The medium can be used for loading directly after washing (the medium should be preserved in 20% methanol if not used).

Application example

Figure 2: Small molecular proteins purified with G75 (super-fine)



Trouble shootings

Problem	Possible cause	Suggestion
Bad resolution between the target peak and impurity peak	Overloading of sample volume.	Decrease the sample volume to 0.5 CV.
	The sample is too viscous.	Dilute the sample properly.
	The flow rate is too fast.	Decrease the flow rate.
	The column is too short.	Choose longer or thinner columns
	Dead space is excessive.	Minimize the dead space between the pipe and joint.
	Bad loading effect of the column.	Reload or use pre-packed column.
	Sample has not been filtered.	Pass the sample through a 0.22 μm or a 0.45 μm filter.
	The medium is too dirty.	Clean the column and equilibrate it again.
	The column is not mounted vertically.	Reload the column.
	Uneven applied temperature.	Constant temperature is recommended.
There is no expected elution peak	The amount of the sample is inconsistent with that before.	Maintain the same sample amount.
	There is ionic interaction between protein and medium.	Maintain the ionic strength of 0.05-0.15M NaCl in buffer.
	There is hydrophobic interaction between protein and medium.	Lowering the ionic strength can minimize the hydrophobic interaction, or reduce the hydrophobic interaction by increasing the pH or adding detergents or organic reagents.
	The sample changes during storage.	Prepare fresh sample.
	Proteins and lipids are precipitated in the chromatography column.	Clean the column or replace a new column.
	There is microbial grow in the media.	No microbial will grow during the operation. The column must be preserved with 20% ethanol when stored.
Elution peak advance	There is a gap in the column.	Reload the column.
	The protein forms dimer or polymer.	Pay attention to the stability of the sample under experimental conditions.

Elution peak delay	There is ionic interaction or hydrophobic interaction between protein and medium.	Maintain the ionic strength of 0.05-0.15M NaCl in buffer.
	Medium, the filter and the top of the column are dirty.	Clean the column and equilibrate it again.
	There is microbial grow in the media.	No microbial will grow during the operation. The column must be preserved with 20% ethanol when stored.
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 applied.
	The column bed is compressed.	Reload the column.
Bubbles appear in the column bed	There is a difference in temperature or residue in the pipe during operation.	Reload the column.
Pressure increase	Sample is turbidity.	Prepare fresh sample.
	Pipe and sieve plate are blocked up.	Clean the pipe, sieve plate, medium, reload the column.