7th Edition, revised in April, 2017

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Focudex G-25 Medium (G25 medium)

Catalog No: E-CM-GF09

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

G-25 medium can be used to purify biological molecules of different molecular weight, which is based on the different crosslinking degree of media, and particle diameter and pore size after swelling. It is mainly applied in sample desalting, buffer exchange and removal of small molecular impurities.

Advantages

1. Easy operation: it can be connected with an injector or a pump, but also can be operated as a gravity column.
2. Quick: the operation can be finished within 5~25 min.
3. Significant effect: desalination rate >98%.
4. High recovery rate: the recovery rate of protein is more than 85%-95%.
5. Easy to be implemented: multiple series and other large size chromatography columns can be loaded according to requirements.

Table 1: Performance index

Matrix	Dextran
Particle size range (dry powder)	45-165 μm
Particle size range (wet gel)	100-300 μm
Swelling degree	4-6 mL/g
Exclusion limit (Mr)	5×10^3 Globular proteins
pH stability	2-13
Void volume	30%CV
Loading amount (volume)	5%-30%CV
Chemical stability	All common buffers
Flow rate	1 mL/min~15 mL/min (5 mL media)
Pressure	≤0.3 MPa
Storage buffer	20% Ethanol (for swelling media)
Storage temperature	4~30℃

Operation (take 5 mL G25 for example)

Note: This product is preserved and shipped in dry powder formality. Swell appropriate amount of dry powder overnight with 8~10 times the dry powder volume of purified water (or swell foe more than 1 hour with boiling water), then use the medium after packing the column.

1. **Wash (water)**

   Wash the media with 2~5 CV (column volume) of purified water with a flow rate of 1.0~10.0 mL/min.

   Note: This operation is used to wash the media (first use) or remove the 20% ethanol in media (reuse).

2. **Equilibration**

   Balance the media with 2~5 CV of equilibrium liquid with a flow rate of 1.0~10.0 mL/min.

   Note: This procedure is used to balance the media. Make sure that the medium is filled with equilibrium liquid.

3. **Sample application**

   Apply the sample with a flow rate of 1.0~10.0 mL/min after centrifugation and filtration (0.45 μm).

   Note: Samples must be centrifuged and filtered and the application volume should be 0.25~1.5 mL.

4. **Elution**

   Elute with 2~5 CV of eluent at with a flow rate of 1.0~10.0 mL/min and collect the eluted solution.

   Note: Collect the eluted solution at suitable stage. The choice of collection time has a certain influence on the recovery rate and desalination rate.

5. **Wash with water**

   Wash the media with 2~5 CV of purified water with a flow rate of 1.0~10.0 mL/min.

   Note: This procedure is used to remove the eluent in media.

6. **Storage**

   Wash the media with 2~5 CV of 20% ethanol with a flow rate of 1.0~10.0 mL/min 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 preferred).

7. **Preparation of buffer**

   Equilibrium liquid/ Eluent: The equilibrium solution and eluent are of the same solution and can be prepared according to requirements of customer.

   Note: It is recommended to add a certain concentration of salt (at least 0.025M) into the target solution to inhibit the ion interaction between the sample and the medium. When the salt concentration is >1.0M, the hydrophobic sample is slightly bound to the medium. When the salt concentration is >1.5M, he medium shrinks slightly.

Note: When operating with gravity column and injector, 5mL/min equals to 100-120 drops/min

Cleaning

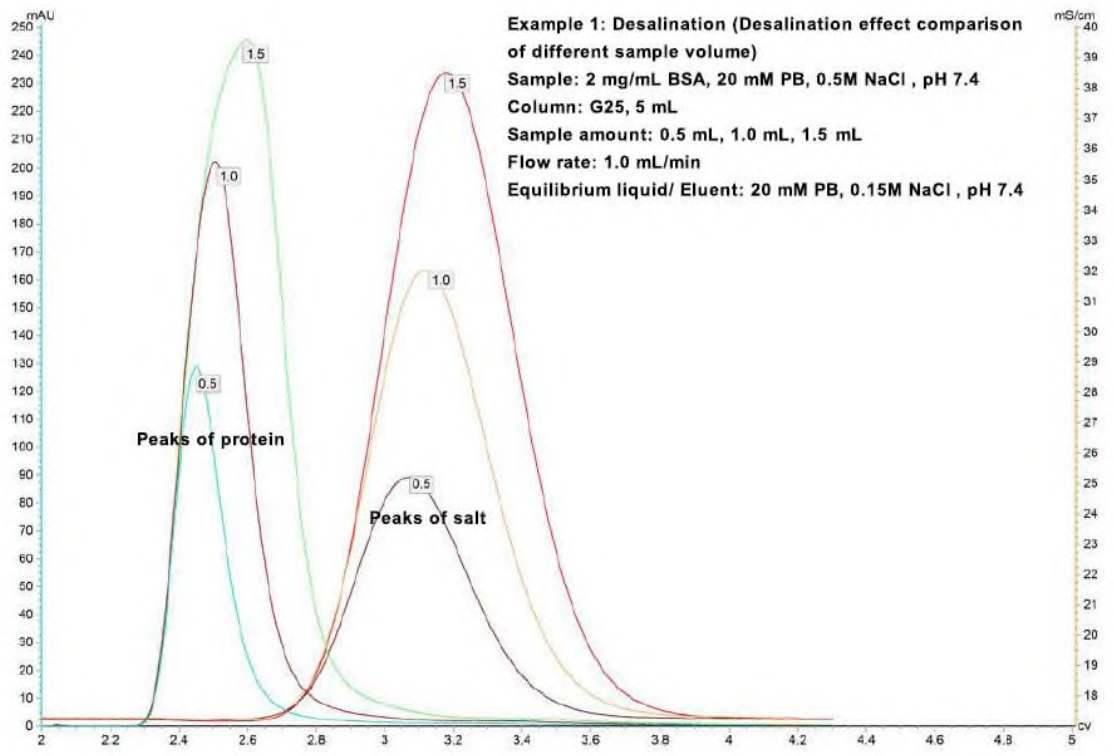
The excellent performance of media (e.g. loading ability, mobility, column efficiency, etc.) can be recovered after cleaning the strong coupling substance (e.g. some strong coupling protein, denatured protein, lipids, etc.).

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 low concentrations of ionic or nonionic detergents (e.g., 0.5%Triton X-100), then wash the media with 5~10 CV of purified water immediately.
   Note: This procedure is used to remove the hydrophobic binding substances.
2. Wash the media with 5~10 CV of 8M urea or 6M guanidine hydrochloride, then wash the media with 5~10 CV of purified water immediately.
   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 0.2M NaOH, then wash the media to neutral with 5~10 CV of purified water immediately.
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).

Application example

Example 1:



Note: The Example 1 shows that the retention time of the target sample varies with the volume of the sample.

Table 2: Relevant data on the application effects of Example 1.

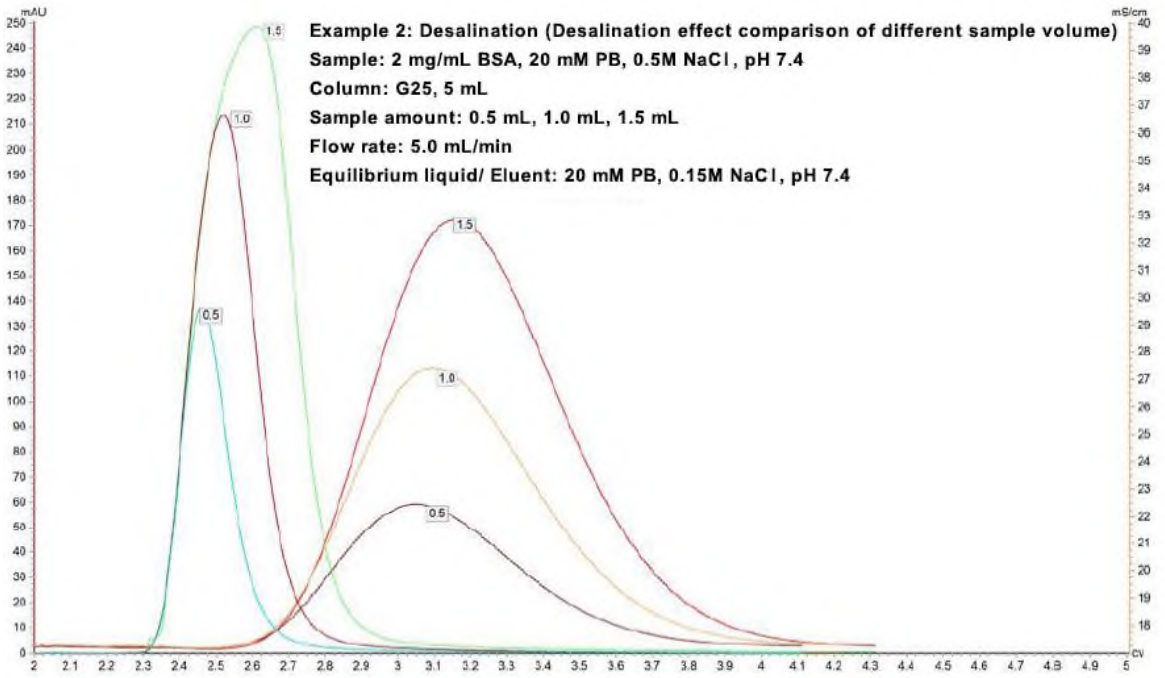
Sample volume (mL)	Collection volume (mL)	Dilution ratio	Desalination rate*	Recovery rate**
0.5	1.2-1.5	2.4-3.0	>99	>85
1.0	1.8-2.0	1.8-2.0	>98	>90
1.5	2.0-2.4	1.3-1.6	>96	>95

Note:

* The effect of desalination mainly depends on the time period of sample collection and the volume of the sample.

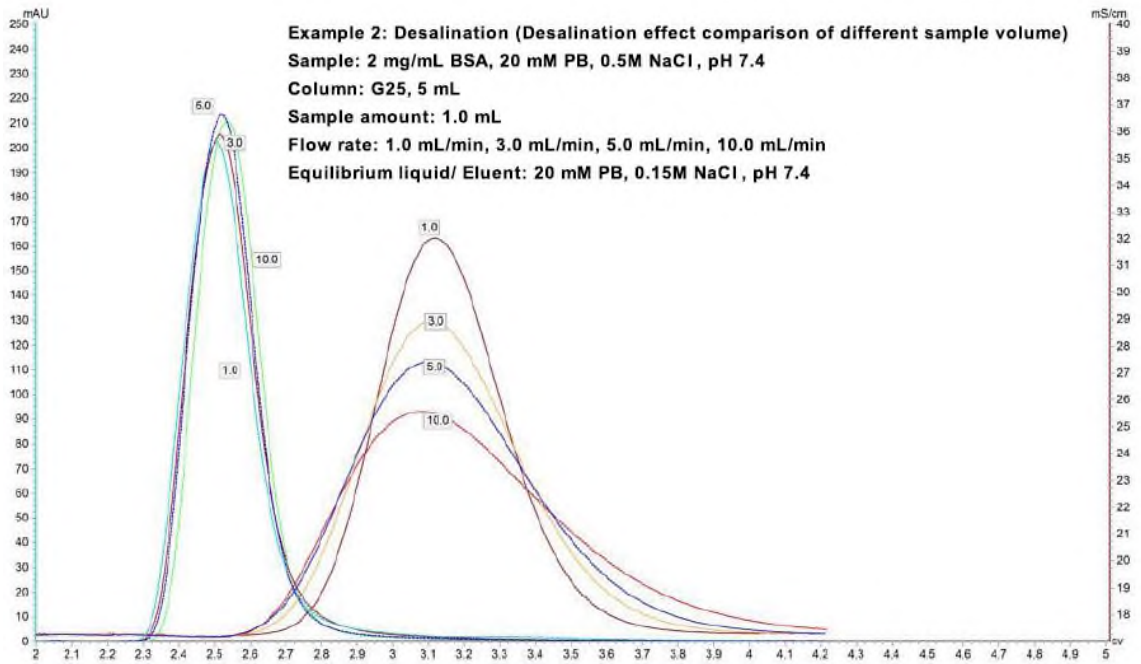
**The recovery rate mainly depends on the property of sample, the time period of the sample collection and the measurement error (when the sample concentration is too low, the measurement error is greater).

Example 2:



Note: Example 1 and Example 2 show that the retention time and concentration of the target sample are not affected by the flow rate.

Example 3:



Note: Example 3 shows that the change of flow rate (within the flow rate range that the medium can tolerate) will not affect the concentration, recovery rate and dilution ratio of the target sample, and will slightly affect the desalination rate.

Trouble shootings

Problem	Possible cause	Suggestion
Low recovery rate	Start collecting too late or stop collecting too early.	Collect the target correctly and timely.
	Hydrophobic interaction between sample and medium.	Increase the flow rate of sample application or decrease the flow rate of elution.
	Sample concentration is too low.	The sample is diluted after elution, and the detection error increases.
	The solubility of the target substance is reduced or gathered near the isoelectric point after desalination.	Properly increase the salt concentration of eluent or adjust the pH to appropriate range.
	Sample has not been pretreated.	Samples must be centrifuged or filtered before loading.
	Bad loading effect of column resin.	Reload the column.
	The top of separation column has a large volume of sample, resulting in the delay of elution.	Reload or re-purchase the column.
	Proteins or lipids accumulate and precipitate in the medium after long-term use, resulting in the delay of elution or residues.	Wash the media timely and effectively.
	There is microbial grow in the media.	Correctly and timely store the media after using.
Poor desalination effect	Stop collecting too late.	Collect the target correctly and timely
	The sample volume is slightly larger.	Properly reduce the volume or concentration of the sample.
	Sample has not been pretreated.	Samples must be centrifuged or filtered before loading.
	Bad loading effect of column resin.	Reload the column.
	The top of separation column has a large volume of sample, resulting in the delay of elution.	Reload or re-purchase the column.
	Proteins or lipids accumulate and precipitate in the medium after long-term use, resulting in the delay of elution or residues.	Wash the media timely and effectively.

	There is microbial grow in the media.	Correctly and timely store the media after using.
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.