GE Healthcare

Instructions 71-7098-00 AD **Gel filtration media**

Sepharose and Sepharose CL

Sepharose™ is a bead-formed agarose-based gel filtration matrix. Sepharose is available with 3 different agarose contents; 2, 4, and 6%, designated Sepharose 2B, Sepharose 4B and Sepharose 6B respectively.

Sepharose CL gels are cross-linked derivatives of Sepharose 2B, Sepharose 4B and Sepharose 6B. The cross-linked form of Sepharose is chemically and physically more resistant than Sepharose itself, offering the same selectivity with better flow characteristics. Cross-linked Sepharose gels are resistant to organic solvents and are thus the choice for separations in organic solvents.

Both Sepharose and Sepharose CL have broad fractionation ranges which makes them suitable for characterizing or cleaning-up samples containing components of diverse molecular weight.

Sepharose™

Table 1. Gel characteristics

Sepharose	2B	4B	6B	CL–2B	CL–4B	CL–6B
% agarose	2	4	6	2	4	6
Optimal MW separation range (globular proteins)	$70x10^3$–$40x10^6$	$70x10^3$–$20x10^6$	$10x10^3$–$4x10^6$	$70x10^3$–$40x10^6$	$70x10^3$–$20x10^6$	$10x10^3$–$4x10^6$
Bead size range (µm)	60–200	45–165	45–165	60–200	45–165	45–165
Recommended linear* flow rate (cm/h)	10	11.	5	14	15	26 30
pH stability** long termand						
working	4–9	4–9	4–9	3–13	3–13	3–13
short term	4–9	4–9	4–9	2–14	2–14	2–14
Chemical stability	Stable to all solutions commonly used in gel filtration including 8 M Urea and 6 M guanidine hydrochloride.			Stable to all solutions commonly used in gel filtration including 8 M Urea and 6 M guanidine hydrochloride. Also stable in organic solvents such as ethanol, DMF, THF, acetone, DMS, chloroform, dichloromethane, dichloroethane, pyridine, triethyl phosphate and acetonitrile.		
Physical stability	Negligible volume variation due to changes in pH or ionic strength					
Sterilization	————-Chemical————-			Autoclavable, 20 min at 120 °C in pH 7		

* Linear flow rate = $\frac{\text{volumetric flow rate } (cm^3/h)}{\text{column cross-sectional area } (cm^2)}$

** The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the gel is stable over a long period of time without adverse effects on its subsequent chromatographic performance. pH stability, short term refers to the pH interval for regeneration and cleaning.

Contents

1. Preparing the gel

Sepharose and Sepharose CL are supplied pre-swollen in 20% ethanol. Prepare a slurry with eluent buffer in a ratio of 75% settled gel to 25% buffer. Degas the gel slurry before packing.

The eluent buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

2. Packing Sepharose and Sepharose CL gels

The column packing method described below has two steps. With the exception of Sepharose 2B, the second step should be performed at the constant pressure given in Table 2.

Resolution increases with increased bed height in gel filtration. It is therefore preferable to choose a bed height of at least 60 cm.

3. Materials

Instruments needed for packing

Pump: P-50, P-900 or similar pump

Recommended columns: XK columns series from GE Healthcare with one or two adaptors.

Packing reservoir (empty XK glasstube of same dimension as column)
Packing connector

Table 2. Recommended packing flow rates and pressures

Gel	Step 1 Flow rate (cm/h)	Step 2 Flow rate (cm/h)	Step 2 Pressure (MPa, bar, psi)
Sepharose 2B	15	5	–
Sepharose 4B	15	–	0.018, 0.18, 2.6
Sepharose 6B	30	–	0.025, 0.25, 3.6
Sepharose CL-2B	30	–	0.020, 0.20, 2.8
Sepharose CL-4B	30	–	0.025, 0.25, 3.6
Sepharose CL-6B	30	–	0.045, 0.45, 6.4

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. Prepare the gel as described above and de-gas the gel slurry.
3. Mount a packing reservoir, using the packing connector, at the top of a column and rinse with distilled water.
4. Insert an end piece or an adaptor at the bottom of the column.
5. Mount the column with the packing reservoir vertically on a laboratory stand. Eliminate air from the column dead spaces by flushing the end piece or adaptor with eluent or distilled water. Make sure no air has been trapped under the net.
   Close the column outlet with approximately one centimeter of eluent remaining in the column.
6. Pour the slurry in a single operation. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles. Fill the reservoir to the top with eluent or distilled water.
7. Screw on the reservoir cap tightly and connect it to the pump.
8. Open the bottom outlet of the column and start the pump at the flow given for step 1 in Table 2.
9. Pack the gel until the gel bed has reached a constant height.
10. Stop the pump, close the column outlet and remove the packing connector and reservoir. This is most easily done by first removing the column from the stand and then unscrewing the reservoir over a sink.

11. Re-mount the column on the stand. Carefully fill the rest of the column with eluent to form an upward meniscus.
12. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
13. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side ofthe column should be turned in all directions during this procedure to ensure that air is removed.
14. Lock the adaptor in position on the gel surface. Open the column outlet and apply a constant pressure (flow for Sepharose 2B) given for step 2 in Table 2, until the gel bed has reached a constant height.
15. Mark on the column the position of the bed surface, stop the pump, close the column outlet and adjust the adaptor to the bed surface and then push the adaptor a further 3–4 mm.

The column is now ready to use.

4. Performance testing of packed columns

To check the quality of the column packing, an efficiency test should be performed to determine the theoretical plate number and peak asymmetry factor.

Eluent: Distilled water
Sample: 2% (v/v) acetone in distilled water

The column is tested by pumping 200 µl of acetone (20mg/ml) through the column at the recommended linear flow given in Table 1. The plate number is calculated as in Figure 1, using the formula:

$N/m = 5.54 (V_R/W_h)^2 \times 1000/L$

and the peak as symmetry factor (A_S) by the formula:

$A_S = b/a$ (see Figure 1)

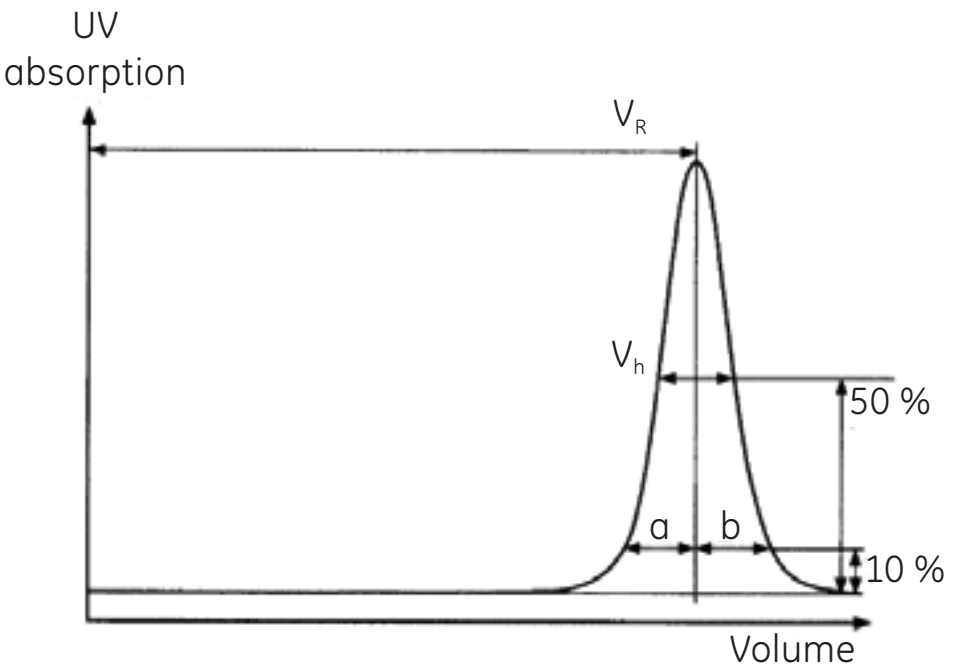

Fig 1. Example showing results obtained from the column evalution method described above.

If the column is packed according to the instructions described above typical values obtained should be:

Gel	Number of theoretical plates per meter	Peak asymmetry factor
Sepharose 2B	> 2 000	0.7–1.3
Sepharose 4B	> 3 000	0.7–1.3
Sepharose 6B	> 3 000	0.7–1.3
Sepharose CL–2B	> 3 000	0.7–1.3
Sepharose CL–4B	> 3 000	0.7–1.3
Sepharose CL–6B	> 3 000	0.7–1.3

5. Separation conditions

It is recommended to use a buffer with an ionic strength of 0.15 or greater to avoid any unwanted ionic interactions between the solute molecule and the matrix.

For recommended flow rates for respective Sepharose gel, please refer to Table 1. The lower the flow rate the better resolution.

6. Eluent and sample preparation

To avoid clogging of column filters, it is recommended to filter or centrifuge the sample to get rid of particulate matter.

7. Column equilibration

Before applying the sample, equilibrate the column with at least two column volumes of the eluent to be used in the separation, or until the baseline is stable. Longer equilibration may be needed with detergent solutions. Equilibration is not needed between runs with the same eluent.

8. Sample application

Recommended sample volumes is 2–5% of the total bed volume.

The sample can be applied via sample applicators SA-5, SA-50 or by using sample loops with valves LV-4 or SRV-4.

9. Regeneration

Regeneration is normally performed by washing with 2–3 column volumes of buffer, followed by re-equilibration in the new buffer (if changing conditions).

In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed using the cleaning procedure described below.

10. Cleaning

Some observations which indicate that column cleaning is necessary;

- increased back-pressure
- colour change at the top of the column
- reduced resolution
- a space between the upper adaptor and the gel surface

If increased back-pressure is observed, check for stoppages in valves, tubing etc. before starting the column cleaning procedure.

Remove precipitated proteins, non-specifically bound proteins and lipoproteins by washing the column with one column volume of 0.5 M NaOH at the recommended linear flow given in Table 1.

Remove strongly non-specifically bound proteins, lipoproteins and lipids by washing the column with two column volumes of a non-ionic detergent solution, e.g. 0.1% Triton™ X-100, followed by at least 2–3 column volumes of eluent buffer.

The cleaning procedures given above can also be performed with the gel on a Buchner funnel.

11. Sanitization

Sanitization reduces microbial contamination of the gel to a minimum.

Wash the column with 0.5 M NaOH at the recommended flow rate, given in Table 1.

Re-equilibrate the column with 3–5 bed volumes of sterile buffer.

12. Storage

Store the gel, packed or unpacked, at +4 to +8 °C in the presence of a bacteriostatic agent, e.g. 20% ethanol.

13. Ordering Information

Designation	Pack size	Code No.
Sepharose 6B	1 L	17-0110-01
Sepharose 6B	10 L	17-0110-05
Sepharose 4B	1 L	17-0120-01
Sepharose 4B	10 L	17-0120-05
Sepharose 2B	1 L	17-0130-01
Sepharose 2B	10 L	17-0130-05
Sepharose CL-6B	1 L	17-0160-01
Sepharose CL-6B	10 L	17-0160-05
Sepharose CL-4B	1 L	17-0150-01
Sepharose CL-4B	10 L	17-0150-05
Sepharose CL-2B	1 L	17-0140-01
Sepharose CL-2B	10 L	17-0140-05

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