GE Healthcare

Instructions 56-1190-97 AD **Gel Filtration**

Sephadex LH-20

Sephadex™ LH-20 is prepared by hydroxypropylation of Sephadex G-25, a bead-formed dextran medium, and has been specifically developed for gel filtration of natural products, such as steroids, terpenoids, lipids and low molecular weight peptides, in organic solvents. Characteristics of Sephadex LH-20 are listed in Appendix B, Table 1.

Sephadex™

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1. Preparing the medium suspension

Sephadex LH-20 is supplied as a dry powder and must be swollen before use. During swelling excessive stirring should be avoided as it may break the beads. Do not use magnetic stirrers.

1. Swell the medium for at least 3 hours in an excess of the solvent to be used in the separation at room temperature.

The extent of swelling will depend upon the solvent system to be used. The table below should be used to calculate the amount of dry medium required to give the required bed volume.

2. Prepare a media slurry in a ratio of 75% settled medium to 25% solvent. Fine particles can be removed by decanting at this time.

Approximate bed volumes (ml media/g dry powder) of Sephadex LH-20 in different organic solvents are given in the table on page 4.

Solvent (ml/g dry powder)	Approx. bed volume
Dimethyl sulphoxide	4.4-4.6
Pyridine	4.2-4.4
Water	4.0-4.4
Dimethylformamide	4.0-4.4
Saline	3.8-4.2
Methanol	3.9-4.1
Ethylene dichloride	3.8-4.1
Chloroform[1]	3.8-4.1
Propanol	3.7-4.0
Ethanol[2]	3.6-3.9
Isobutanol	3.6-3.9
Formamide	3.6-3.9
Methylene dichloride	3.6-3.9
Butanol	3.5-3.8
Isopropanol	3.3-3.6
Tetrahydrofuran	3.3-3.6
Dioxane	3.2-3.5
Acetone	2.4-2.6
Acetonitrile[3]	2.2-2.4
Carbon tetrachloride[3]	1.8-2.2
Benzene[3]	1.6-2.0
Ethyl acetate[3]	1.6-1.8
Toluene[3]	1.5-1.6

[1] Containing 1% ethanol
[2] Containing 1% benzene
[3] Solvents giving a bed volume of less then about 2.5 ml/g dry media are generally not useful

2. Packing Sephadex LH-20

1. Equilibrate all material to room temperature.
2. Resuspend and pour the media slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column prevents the introduction of air bubbles. Fill the reservoir to the top with buffer. Screw on the reservoir top tightly, and connect it to a pump. Open the column outlet.
3. Open the bottom outlet of the column and begin packing at 300 cm/h until the media bed has reached a constant height. The column should be packed at as high pressure as is possible without deforming the beads (the pressure tolerance of the column is the limity factor, pack at the maximum pressure specified for your column) Stop the pump, close the column outlet and remove the column from the stand. Unscrew and remove the packing reservoir over a sink.
4. Re-mount the column on the stand and carefully fill with buffer to form a meniscus at the top of the column.
5. Wet the upper adaptor by drawing solvent through it using a syringe. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net. Adjust the adaptor O-ring to give a sliding seal on the column wall.
6. Make all tubing connections at this stage. There must be a bubble-free eluent connection between the column and the pump and the column and the sample application system.

7. Slide the adaptor slowly down the column so that any air in the tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
8. Lock the adaptor in position on the medium surface. Open the column outlet and continue packing until the medium bed is stable. Re-position the adaptor on the medium surface as necessary.

Note: The above packing method can be used for packing beds of Sephadex LH-20 in most solvents. In some solvents, e.g. chloroform, Sephadex LH-20 is less dense than the solvent and floats in it. A column equipped with two flow adaptors, e.g. SR 25/45 and SR 25/100, should be used. All the required medium should be poured into the column and drained until there is space enough to insert the second adaptor. Having locked this adaptor in position at the surface of the medium, the chloroform flow is directed upwards. The bed will now be packed against the upper flow adaptor and it can be fixed in that position by slowly pushing the lower adaptor upwards. When moving the adaptor, the column outlet should be closed to avoid compressing the media bed.

The column is now ready for use.

3. Equilibration

Before applying the sample, equilibrate the column with eluent to be used in the separation until the baseline becomes stable (at least two bed volumes). If changing solvents careful attention should be paid to the medium's swelling characteristics in the new solvent and the adaptor(s) repositioned accordingly. Equilibration is not needed between runs with the same eluent.

4. Eluents

To ensure long column life, all buffers should be centrifuged or filtered through a filter (0.45 µm) resistant to the solvent before use.

5. Samples

The sample volume should be in the range of 1–2% of the total bed volume. To ensure long column life, samples should be centrifuged or filtered (0.45 µm, solvent resistant) before use.

6. Elution

The recommended flow rate range is dependent on the application. Flow rates of (1–10 cm/h) are recommended. Generally, the lower the flow rate, the better the resolution.

7. Regeneration

Regeneration is normally performed by washing the column with 2–3 column volumes of eluent, followed by re-equilibration in a new eluent if changing conditions. See Equilibration above.

8. Storage

Dry Sephadex LH-20 should be stored at +4 °C to +30 °C. Packed columns and used medium should be stored at +4 °C to +8 °C with a bacteriostat.

Appendix A

Flow rates

To convert flow rates for columns of different dimensions:

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

Volumes

Since the swelling characteristics of Sephadex LH-20 are solvent dependent all volumes (solvent, eluent, etc. are quoted as bed volumes. To convert volumes for columns of different dimensions, increase or decrease in proportion to the new column bed volume.

$$\text{New volume} = \text{Old volume} \times \frac{\text{New bed volume}}{\text{Old bed volume}}$$

Appendix B

Table 1. Medium characteristics

Exclusion limit (M_r)	4000–5000 (depends on solvent)
Sample loading:	
adsorption mode	depends on resolution required
molecular sizing	< 2% total bed volume
partition mode	< 1% total bed volume
Matrix	Hydroxypropylated, cross-linked dextran
Particle form	Spherical, porous
Particle size range (dry)	18–111 µm
Average diameter (dry)	70 µm
Particle size range (methanol)	27–163 µm
Average diameter (methanol)	103 µm
Max. linear flow rate	720 cm/hour
Rec. linear flow rate	60 cm/hour
pH stability	
working	2–13
cleaning	2–13
Chemical stability	Stable in most aqueous and organic eluent systems. Not stable below pH 2 nor to strong oxidising agents.
Autoclavable	20 min at +121 °C
Operating temperature	+4 to +40 °C
Shelf-life	5 years

9. Ordering Information

Description	Pack size	Code No.
Sephadex LH-20	25 g	17-0090-10
	100 g	17-0090-01
	500 g	17-0090-02

Related Products

Column SR 25/45	19-0879-01
Column SR 25/100	19-0880-01
Column Packing the Movie	18-1165-33
Gel Filtration Handbook; Principles and methods	18-1022-18
Preparative Gel Chromatography on Sephadex LH-20 by H. Henke	18-1113-89

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