GE Healthcare

Instructions for Sephadex Media

Sephadex is a beaded gel prepared by crosslinking dextran with epichlorohydrin.

Its main application is group separations of low and high molecular weight molecules. Desalting is the most common use. Desalting with Sephadex is superior to dialysis because of the considerable time savings, the low dilution factor (which can be as low as 1.4:1), and the high activity recoveries even with very small amounts of sample. Sephadex G-25 is available prepacked in PD-10 columns, which are ideal for rapid, routine work.

Related applications include buffer exchange and the removal of small molecules during the preparation of large biomolecules, such as ampholytes, detergents, radioactive or fluorescent labels, and phenol (during DNA purification).

Table 1. Gel Characteristics

Gel Type	Fractionation Range, Globular Proteins (D)	Fractionation Range, Dextrans (D)	Approx. Bed Volume (mL swelled per g dry)	Approx. Dry Bead Diameter (µm)	Approx. Maximum Operating Pressure (bar)
G-10	≤700	≤700	2–3	40–120	*
G-15	≤1,500	≤1,500	2.5–3.5	40–120	*
G-25 Superfine Fine Medium Coarse	 1,000–5,000 (all grades)	 100–5,000 (all grades)	 4–6 (all grades)	 20–50 20–80 50–150 100–300	*
G-50 Superfine Fine Medium Coarse	 1,500–30,000 (all grades)	 500–10,000 (all grades)	 9–11 (all grades)	 20–50 20–80 50–150 100–300	*
G-75 G-75 Superfine	3,000–80,000 3,000–70,000	1,000–50,000 1,000–50,000	12–15 12–15	40–120 20–50	0.16 0.16
G-100 G-100 Superfine	4,000–150,000 4,000–100,000	1,000–100,000 1,000–100,000	15–20 15–20	40–120 20–50	0.096 0.096
G-150 ** G-150 Superfine	5,000–300,000 5,000–150,000	1,000–150,000 1,000–150,000	20–30 18–22	40–120 20–50	0.036 0.036
G-200 ** G-200 Superfine	5,000–600,000 5,000–250,000	1,000–200,000 1,000–200,000	30–40 20–25	40–120 20–50	0.016 0.016

* The beads behave as rigid spheres and therefore obey Darcy's Law: the flow rate is proportional to the pressure drop over the bed and inversely proportional to the bed height.

** Sephadex G-150, G-150 SF, G-200 & G-200 SF are discontinued products.

Table 2. Swelling Times

Gel Type	Swelling Time, 20° C (hr)	Swelling Time, 90° C (hr)
G-10	3	1
G-15	3	1
G-25 (all grades)	3	1
G-50 (all grades)	3	1
G-75 (all grades)	24	3
G-100, G-100 SF	72	5
G-150, G-150 SF	72	5
G-200 G-200 SF	72	5

Swelling

Sephadex is supplied as a dry powder, and must be allowed to swell in excess buffer before use. Filter all buffers through a 0.22-µm filter to help prevent microbial growth.

1. Weigh out the appropriate amount of dry Sephadex for the required bed volume of your column (Table 1). If packing at the maximum pressure for the gel, choose the lowest bed volume factor (mL/g) for calculating the amount of dry Sephadex.
2. Add enough buffer to equal the total volume of the column plus 30%. Swelling times for the different types of Sephadex are given in Table 2. The process is accelerated by using a boiling water bath.
3. After swelling is complete, decant the supernatant.
4. Add buffer to make a 75% suspension.
5. Degas the suspension before packing.

Packing By Gravity Flow

1. Pour the entire slurry into the column in one portion, taking care not to trap air bubbles.
2. Start the gravity flow to initiate packing.

Packing Using a Flow Adaptor

Good packing is essential to obtain good resolution. Ideally, the column should be packed at the highest pressure possible without deforming the beads.
For Sephadex G-10 – G-50, the beads behave as rigid spheres. The pressure tolerance of the column is the limiting factor: pack at the maximum pressure specified for your column.
For the softer gels, Sephadex G-75 – G-200, more care must be taken to avoid compressing the gel. Do not increase the pressure beyond the values given in Table 1.
With wider columns, slightly reduced maximum operating pressures must be used. Flow rate is inversely proportional to bed height: increasing the bed height will decrease the flow rate, but does not affect the maximum operating pressure.

1. Using a reservoir if necessary, pour the entire slurry into the column in one portion.
2. Start the pump to initiate packing.
3. Once all the gel has sedimented into the column, remove the reservoir.
4. Insert an adaptor and pack at the maximum operating pressure of the gel.
5. Once the gel is thoroughly packed into the column, adjust the flow adaptor to the surface of the gel bed.
6. Pass a further 2–3 column volumes of the buffer to be used in the separation. This stabilizes and equilibrates the bed.
7. Readjust the flow adaptor to the surface of the gel bed.

Cleaning

1. Wash with two column volumes of 0.2 M NaOH or a solution of a nonionic detergent. Washing the more porous Sephadex types (G-50 – G-200) with NaOH should be done outside the column because the gel will swell.
2. Reequilibrate the gel with 2–3 column volumes of buffer before your next experiment. When necessary, the gel can be removed from the column and sterilized by autoclaving at 120 °C, pH 7.

Storage

Store unused gel dry at 2–8 °C.

Store used gel at 2–8 °C in 20% ethanol or in a solution of a microbial growth inhibitor such as 0.002% Hibitane/chlorhexidine or 0.02% sodium azide.

Ordering Information

Product	Pack Size	Product Code
Sephadex G-10	100 g	17-0010-01
Sephadex G-15	100 g	17-0020-01
Sephadex G-25 Superfine	100 g	17-0031-01
Sephadex G-25 Fine	100 g	17-0032-01
Sephadex G-25 Medium	25 g	17-0033-10
Sephadex G-25 Medium	100 g	17-0033-01
Sephadex G-25 Coarse	100 g	17-0034-01
Sephadex G-50 Superfine	100 g	17-0041-01
Sephadex G-50 Fine	100 g	17-0042-01
Sephadex G-50 Medium	100 g	17-0043-01
Sephadex G-50 Coarse	100 g	17-0044-01
Sephadex G-75	100 g	17-0050-01
Sephadex g-75 Superfine	100 g	17-0051-01
Sephadex G-100	100 g	17-0060-01
Sephadex G-100 Superfine	100 g	17-0061-01