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TECHNICAL INFORMATION

Catalog Number: 195251, 195252, 195253, 195254, 195255, 195578, 195579, 195580, 195581, 195582, 195583, 195588, 195589

Sephadex®

Sephadex® is a registered trademark of Amersham-Pharmacia

– Sephadex® G-25 is the first choice for desalting - faster and safer than dialysis.
– Sephadex® G-25 is ideal for buffer exchange and for removal of free label, e.g. 32P, 125I, 35S.

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.
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

				Fractionation Range (M_r)			
Gel	**Working pH Range**	**Particle Size Range Wet Bead (um)**	**Bed Volume (ml/g) in distilled water**	**Dextrans**	**Globular Proteins**	**Max. Flow Rate (ml/min)**	**Max. Oper. Pressure (bar)**
Sephadex® G-10	2-13	55-165	2-3	$< 7 \times 10^2$	$< 7 \times 10^2$	D	D
Sephadex® G-25							
Coarse	2-13	170-520	4-6	1×10^2 - 5×10^3	1×10^3 - 5×10^3	D	D
Fine		35-140					
Medium		85-260					
Superfine		17-70					
Sephadex® G-50							
Course	2-10	200-610	9-11	5×10^2 - 1×10^4	1.5×10^3 - 3×10^4	D	D
Fine		40-160					
Medium		100-300					
Superfine		20-80					
Sephadex® G-75	2-10	90-280	12-15	1×10^3 - 5×10^4	3×10^3 - 8×10^4	6.4	0.15
Sephadex® G-75 Superfine		25- -90			3×10^3 - 7×10^4	1.5	0.15
Sephadex® G-100	-	40-120 (dry bead)	15-20	1000-100000	4000-150000	-	0.096

Sephadex® G-100 Superfine	-	20-50 (dry bead)	15-20	1000-100000	4000-100000	-	0.096
D: The beads behave as rigid spheres obeying Darcy's Law							

Table 2. Swelling Times

Gel Type	Swelling Time, 20°C (hr)	Swelling Time, 90°C (hr)
G-10	3	1
G-25 (all grades)	3	1
G-50 (all grades)	3	1
G-75 (all grades)	24	3
G-100 and G-100 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 um filter to help prevent microbial growth.

– Weigh out the appropriate amount of dry Sephadex® for the required bed volume of your column (Table 1). If packed at the maximum pressure for the gel, choose the lowest bed volume factor (ml/g) for calculating the amount of dry Sephadex®.
– 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.
– After swelling is complete, decant the supernatant.
– Add buffer to make a 75% suspension.
– Degas the suspension before packing.

Packing By Gravity Flow

– Pour the entire slurry into the column in one portion, taking care not to trap air bubbles.
– 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 and up, 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.

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

Cleaning

– 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 and up) with NaOH should be done outside of the column because the gel will swell.
– Re-equilibrate 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 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.

Availability:

Catalog Number	Description	Size
195251	Sephadex® G-10, Coarse Fractionation Range (MW)	10 g 50 g 100 g
195578	Sephadex® G-25, Coarse Fractionation Range (MW)	10 g 50 g 100 g
195254	Sephadex® G-25, Medium Fractionation Range (MW)	10 g 50 g 100 g
195252	Sephadex® G-25, Fine Fractionation Range (MW)	10 g 50 g 100 g
195253	Sephadex® G-25, Super-Fine Fractionation Range (MW)	10 g 50 g 100 g
195579	Sephadex® G-50, Coarse Fractionation Range (MW)	10 g 50 g 100 g
195580	Sephadex® G-50, Medium Fractionation Range (MW)	10 g 50 g 100 g
195581	Sephadex® G-50, Fine Fractionation Range (MW)	10 g 50 g 100 g
195582	Sephadex® G-50, Super-Fine Fractionation Range (MW)	10 g 50 g 100 g
195588	Sephadex® G-75	10 g 50 g 100 g
195589	Sephadex® G-75, Super-Fine Fractionation Range (MW)	10 g 50 g 100 g
195255	Sephadex® G-100	10 g 50 g 100 g
195583	Sephadex® G-100, Super-Fine Fractionation Range (MW)	10 g 50 g 100 g