3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

SEPHAROSE AND SEPHAROSE CL GEL FILTRATION MEDIA

Exact replacement for Product Code 84963

The information below is abstracted, for customer convenience, from a supplier technical manual and a supplier information sheet. Complete copies of supplier data are available upon request from Sigma Research Technical Service.

SEPHAROSE	SEPHAROSE CL
D-Galactose 3,6-Anhydro-L-Galactose Partial structure of Sepharose CL	D-Galactose 3,6-Anhydro-L-Galactose Partial structure of Sepharose CL
CHARACTERISTICS	
- Broad fractionation range - High exclusion limits - Negligible non-specific adsorption - Appearance: white suspension	- Broad fractionation range - High exclusion limits - Negligible non-specific adsorption - Excellent chemical and physical stability - Appearance: white suspension
PRODUCT DESCRIPTION	
Sepharose is a beaded agarose gel filtration medium with a broad fractionation range. Three different agarose contents are available: 2%, 4% and 6%, designated 2B, 4B and 6B, respectively. As agarose concentration increases porosity decreases, thus increasing rigidity and altering the fractionation range; nucleic acids and polysaccharides with molecular weights up to -4X10^7 can be separated on Sepharose 2B.	Sepharose CL is a cross-linked derivative of Sepharose, prepared by reacting Sepharose with 2,3-dibromopropanol under strongly alkaline conditions. After cross-linking, the gel is desulfated by alkaline hydrolysis under reducing conditions. The resulting crosslinked polysaccharide chains have a very low content of ionizable groups and better chemical and physical resistance than Sepharose, as well as improved flow properties. Porosity, however, is comparable to that of Sepharose. Three different agarose contents are available: 2%, 4% and 6%, designated CL-2B, CL-4B and CL-6B,

				respectively.			
Property	**2B**	**4B**	**6B**	**Property**	**CL-2B**	**CL-4B**	**CL-6B**
MW Range:				**MW Range:**			
Globular Proteins	$7X10^4$-$4X10^7$	$6X10^4$-$2X10^7$	$1X10^4$-$4X10^6$	**Globular Proteins**	$7X10^4$-$4X10^7$	$6X10^4$-$2X10^7$	$1X10^4$-$4X10^6$
Dextrans	$1X10^5$-$2X10^7$	$3X10^4$-$5X10^6$	$1X10^4$-$1X10^6$	**Dextrans**	$1X10^5$-$2X10^7$	$3X10^4$-$5X10^6$	$1X10^4$-$1X10^6$
DNA exclusion limit	-1352 base pairs	-872 base pairs	-45-165 base pairs	**DNA exclusion limit**	-1353 base pairs	-872 base pairs	-45-165 base pairs
Diameter (wet bead)	-60-200 µm	-45-165 µm	-45-154 µm	**Diameter (wet bead)**	-60-200 µm	-45-165 µm	-45-165 µm
pH Range	4-9	4-0	4-9	**pH Range**	3-14	3-14	3-14
Max. Pressure*	40 cm H_2O	80 cm H_2O	200 cm H_2O	**Max. Pressure***	50 cm H_2O	120 cm H_2O	>200 cm H_2O
Max. Volumetric Flow Rate*	0.83 mL/min	0.96 mL/min	1.16 mL/min	**Max. Volumetric Flow Rate***	1.25 mL/min	2.17 mL/min	2.5 mL/min
Max. Linear Flow Rate*	10 mL/cm^{2}h	11.5 mL/cm^{2}h	14 mL/cm^{2}h	**Max. Linear Flow Rate***	15 mL/cm^{2}h	26 mL/cm^{2}h	30 mL/cm^{2}h

STABILITY

Sepharose melts upon heating to 40EC, cannot be autoclaved, and the bead structure may be damaged upon freezing. Due to the presence of 3,6-anhydro-L-galactose, the matrix is resistant to biological degradation. Sepharose is stable in aqueous (including saline) solutions at pH 4-9. Use of dissociation media such as guanidine hydrochloride and urea, chaotropic salts such as KSCN, and oxidizing agents is not advisable because these reagents may disrupt the hydrogen bonds which stabilize the matrix. Sterilization can be done chemically (e.g., by treatment with DEPC).

Sepharose CL is stable in aqueous media at pH 3-14, and in organic solvents. Dissociating media and chaotropic salts may be used, but oxidizing conditions should be avoided in order to preserve the identity of the constituent sugar residues. Sepharose CL may be autoclaved repeatedly at 120EC and pH 7. The matrix is resistant to biological degradation. Flow rates may be up to 50% higher than on Sepharose with an equivalent fractionation range.

USAGE AND REGENERATION

Sepharose and Sepharose CL are supplied pre-swollen as suspensions in distilled water. Before packing a column, dilute the required amount of gel with starting buffer to form a thick slurry, about 75% of which is settled resin, then degas the slurry. The maximum operating pressures (shown above) should never be exceeded. Pass 2-3 column volumes (CV) of eluent through the gel to equilibrate the bed. Elution may be achieved by gravity feed or through the use of a peristaltic pump; more consistent flow rates and more reproducible separations are obtained with a pump. Sepharose contains a small number of ionic sulfate and carboxyl groups which may cause adsorption of basic proteins at low ionic strengths. Therefore, eluents with

<table>
<tr><td colspan="2">ionic strengths exceeding 0.02 M are sometimes necessary (for example, tRNA species have been resolved in high concentrations of ammonium sulfate, and DNA has been separated from RNA in 1.5 N NaCl). The gels can be cleaned as indicated below and stored at 4-8EC in a suitable antimicrobial agent (e.g., 20% ethanol) for indefinite time periods.</td></tr>
<tr><td>Sepharose should be cleaned in the column or batchwise with a non-ionic detergent solution.</td><td>Sepharose CL can be cleaned in the column or batchwise. Wash with at least 1 CV of 0.5 N NaCl in 0.1 N NaOH (a step which also sterilizes the resin), then with 10 CV of water (or until the eluent is at neutral pH).</td></tr>
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