G-Sep™ 25 & 50

Superfine, Fine & Medium resins

(Cat. # 786-1390, 786-1391, 786-1392, 786-1393, 786-1394, 786-1395, 786-1396, 786-1397, 786-1398, 786-1399, 786-1400, 786-1401, 786-1402, 786-1403, 786-1404, 786-1405, 786-1406, 786-1407)

INTRODUCTION

G-Sep™ 25 and G-Sep™ 50 are gel filtration resins comprised of ultrapure cross-linked dextran for laboratory applications such as desalting of protein, carbohydrate and DNA preparations, as well as easy buffer exchanges and sample clean up. The G-Sep™ resins exhibit high selectivity, superb resolution, low non-specific adsorption and robust chemical stability.

- Quickly desalts, removes contaminants and transfers to a new buffer in a single step.
- Excellent recovery and minimum sample dilution
- Available in prepacked SpinOUT™ Desalting columns for fast and convenient desalting

Molecules purified with G-Sep™ resins are separated according to size. Smaller molecules (salts, free labels, and other impurities) pass significantly slower through the column than larger molecules. The degree of cross-linking of the dextran determines the extent to which macromolecules can permeate the beads (molecular fractionation range). Large molecules whose molecular weights are above the molecular fractionation range are excluded from entering the beads and leave the column first. The smaller sized molecules within the molecular fractionation range enter the beads and are eluted last.

Two different G-Sep™ resins are available, G-Sep™ 25 and G-Sep™ 50. They differ in their degree of cross-linking and hence in their degree of swelling and molecular fractionation range. G-Sep™ 25 is better suited for smaller molecules and G-Sep™ 50 is better suited for larger molecules.

- G-Sep™ 25 molecular weight cut-off (MWCO): 5 kD for proteins and 10 bases for nucleic acids
- G-Sep™ 50 molecular weight cut-off (MWCO): 25 kD for proteins and 20 bases for nucleic acids

G-Sep™ 25 and G-Sep™ 50 resins are available in three different particle sizes (Superfine, Fine and Medium). The Medium particle size is preferred for group separations at process scale where high flow rates and low operating pressures are required. For most routine laboratory work including preparative separations, Fine and Superfine particle sizes are appropriate. The smaller particle sizes of Fine and Superfine give shorter diffusion distances and allow for highly efficient separations at high flow rates. For micropreparative work using very small sample volumes, Superfine will be the particle size of choice.

Buffer and pH effects on resolution are minimal and purified biomolecules are not significantly diluted (1.5-fold) when processed using G-Sep™ 25 and G-Sep™ 50.

G-Sep™ 25 and G-Sep™ 50 are autoclavable at 121°C, pH 7 for 30 minutes and are stable in all commonly used buffers, including: 0.2M NaOH; 0.2M HCl; 1M acetic acid; 8M urea; 6M guanidine HCl; 1% SDS, 24% Ethanol; 30% Propanol; and 30% Acetonitrile.

ITEM(S) SUPPLIED

Cat#	Description	Size
786-1390	G-Sep™ 25 Superfine	100g
786-1391		500g
786-1392		1kg
786-1393	G-Sep™ 25 Fine	100g
786-1394		500g
786-1395		1kg
786-1396	G-Sep™ 25 Medium	100g
786-1397		500g
786-1398		1kg
786-1399	G-Sep™ 50 Superfine	100g
786-1400		500g
786-1401		1kg
786-1402	G-Sep™ 50 Fine	100g
786-1403		500g
786-1404		1kg
786-1405	G-Sep™ 50 Medium	100g
786-1406		500g
786-1407		1kg

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store at 4°C. DO NOT FREEZE.

SPECIFICATIONS

G-Sep™ G-25

	Superfine	Fine	Medium
BioProcess resin	Yes		
Matrix	Cross-linked dextran		
Wet bead size	35–90 µm	35-140 µm	38–235 µm
Dry bead size	20-50 µm	20-80 µm	50-150 µm
Water regain	2.15 – 2.25 mL/g		
Swelling	4 – 6 mL/g		
MWCO (size exclusion)	below 5000 Da		
Fractionation range Mr Globular proteins	1000 - 5000 Da		
pH Stability	2.0 to 13.0		
Pressure Flow Specification	>11 cm/h, pressure drop cm H2O/bed height=2, bed height 30 cm, 2.6 cm i.d.		

G-Sep™ 50

	Superfine	Fine	Medium
BioProcess resin	Yes		
Matrix	Cross-linked dextran		
Wet bead size	40 – 100 µm	40 – 160 µm	100 – 300 µm
Dry bead size	20 – 50 µm	20 – 80 µm	50 – 150 µm
Water regain	4.80 – 5.20 mL/g		
Swelling	9 – 11 mL/g		
MWCO (size exclusion)	below 25,000 Da		
Fractionation range Mr Globular proteins	1,000 – 30,000 Da		
pH Stability	2.0 to 13.0		
Pressure Flow Specification	min 60 cm/h, pressure drop cm H2O/bed height=15, bed height 10 cm, column 5 cm i.d.		

PREPARING THE MEDIUM

G-Sep™ 25 and G-Sep™ 50 are supplied in a solution containing 20% ethanol.

Prepare a 75% slurry using a binding buffer/eluent of choice. The slurry will be 75% settled resin and 25% buffer. It is recommended to degas the slurry before packing.

PACKING G-SEP™ 25 AND G-SEP™ 50

1. Equilibrate all material to room temperature.
2. De-gas the slurry
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the gel slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate is typically employed during packing.
7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adapter

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
2. Insert the adaptor into the top of the column at an angle, taking care not to trap air under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubing 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.
5. Lock the adapter in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adapter on the medium surface as necessary.
6. The column is now packed and equilibrated and ready for use.

OPERATION

Equilibration

Equilibrate the column with the starting buffer when the pH and/or conductivity of the effluent is the same as the starting buffer.

Sample preparation

Before application the sample should be centrifuged or filtered through a 0.45µm filter to remove any particulate matter. Recommended sample volume is 2-5% of the total bed volume.

Elution

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 cross-linked dextran.

Regeneration

After every run, elute reversibly bound material with low ionic strength buffer, and wash with H_2O and starting buffer.

Cleaning-in-place (CIP)

Remove precipitated proteins and hydrophobically bound proteins or lipoproteins: Wash with 0.5 M NaOH and immediately rinse with eluent buffer.

Lipids and very hydrophobic proteins: Wash the column with non-ionic detergent, followed by at least 2-3 column volumes of eluent buffer.

Sanitization

Wash the column with 0.5M NaOH for 30-60 min. Sanitization is the use of chemical agents to inactivate microbial contaminants in the form of vegetative cells; it also helps to maintain a high level of both process hygiene and process economy.

Storage

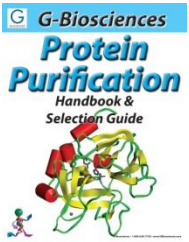
G-Sep™ 25 and G-Sep™ 50 should be stored in the salt form in a buffer containing 20% ethanol. Recommended storage at 4°C to 30°C. Do not freeze.

Shelf life

5 years

RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.



http://info2.gbiosciences.com/complete-protein-purification-handbook

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