G-Sep™ Size Exclusion Columns

(Cat. # 786-1297, 786-1298, 786-1300, 786-1301, 786-1302, 786-1304)

INTRODUCTION

Size Exclusion Chromatography or gel filtration is a widely used technique for separation of proteins and other molecules based on their sizes. G-Sep™ Size Exclusion Columns are available in there column formats including 11/30, 16/60 and 26/60 and with two separation range, 3 kDa to 70 KDa and 6 kDa to 600 kDa. G-Sep™ Size Exclusion Columns have non-adjustable end adaptors.

G-Sep™ Size Exclusion Column resin design is made to offer high recovery and high selectivity. The resin offers high resolution, high selectivity, high recovery and is highly scalable. In addition, the resin has excellent physical and mechanical stability and low on cost. The resin is highly cross-linked polysaccharide composite of dextran and agarose and is stable to most of the aqueous and organic solvents used in chromatography.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1297	G-Sep™ Size Exclusion Column (11 mm ID; 30 cm length, 3-70 kDa HR resin)	1 Column
786-1298	G-Sep™ Size Exclusion Column (16 mm ID; 60 cm length, 3-70 kDa HR resin)	1 Column
786-1300	G-Sep™ Size Exclusion Column (26 mm ID; 60 cm length, 3-70 kDa HR resin)	1 Column
786-1301	G-Sep™ Size Exclusion Column (11 mm ID; 30 cm length, 6-600 kDa HR resin)	1 Column
786-1302	G-Sep™ Size Exclusion Column (16 mm ID; 60 cm length, 6-600 kDa HR resin)	1 Column
786-1304	G-Sep™ Size Exclusion Column (26 mm ID; 60 cm length, 6-600 kDa HR resin)	1 Column

STORAGE CONDITIONS

G-Sep™ Size Exclusion Column are supplied at ambient temperature. Upon arrival, store it at 4°C, DO NOT FREEZE. This product is stable for 1 year. The resin in the column should be stored in 20% ethanol at 4°C after use.

ADDITIONAL ITEMS REQUIRED

- Sample containing molecules to be separated using the G-Sep™ Size Exclusion Column.
- Equilibration/starting buffer and elution buffer
- Operation Unit: Chromatography system
- 20% ethanol.

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SECIFICATIONS

Features	G-Sep™ Size Exclusion Column, 3 -70kDa HR resin	G-Sep™ Size Exclusion Column, 6-600kDa HR resin
Matrix	Highly cross-linked polysaccharide composite of Dextran and Agarose	
Particle size (µm)	35 µm (in the range of 20- 50 µm)	
Column body maximum pressure	6 Bar, 0.6 MPa (3-70 Columns)	5 Bar, 0.5 MPa (6-600 Columns)
Maximum resin pressure	4 Bar	3 Bar
Operating Flow velocity	10-50 cm/hr; 0.15-0.8 ml/min (11/30 Columns), 0.30-1.6 ml/min (16/60 Columns), 0.80-4.4 ml/min (26/60 Columns)	
pH stability	2-14 (short term) and 3-12 (long term)	
Working temperature	+4°C to +30°C	
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH, 6 M guanidine hydrochloride, 8M Urea, 30% isopropanol, 20% ethanol (concentration of alcohol should not exceed 30% v/v)	
Storage	Storage buffer with 0.02% sodium azide or 20 % ethanol	
Precaution	Avoid oxidizing agents	

INSTRUCTION FOR USE

Instructions for handling and use of G-Sep™ Size Exclusion Columns.

1. The column is sealed at both ends by mildly pressurized syringes filled with 20% ethanol.
2. Remove the column from packing material and always hold the column from the middle.
   NOTE: *Do not hold the column by syringe side.*
3. Check the labeling for orientation; firmly clamp the column at two points in a vertical position using an appropriate support.
4. Disconnect the top syringe first by gently unwrapping the rubber band from the top syringe.
5. Hold the column body from middle and unscrew the 1/16” tubing adaptor from the top of the column.
   NOTE: *Keep the syringe-tubing aside for later use.*
6. Connect the chromatography system to the top of column via its female 1/16” thread connection.
   NOTE: *For M6 connectors tubing connection, a 1/16” male thread-M6 female thread connector is required.*
7. Keep the flow rate at approximately 0.5 ml/min and fill the thread cavity with liquid before tightening the connection to avoid air bubbles.
8. Unwrap the rubber band from bottom syringe gently.

9. Hold the column and unscrew the 1/16”-tubing adaptor from the bottom of the column.
   NOTE: *Keep the syringe-tubing aside for later use.*
10. Connect the bottom of the column to the chromatography system.

OPERATION

Equilibration

1. Equilibrate the column by passing at least 2 column volumes (CV) of equilibration/starting buffer at a flow rate of 1 ml/min for 16 mm column and 2.5 ml/min for 26 mm column before loading sample. Equilibrate until stable baseline is reached.
2. Re-equilibration between runs is not necessary. In case denatured proteins or lipids are not eluted with one column volume, the cleaning-in-place procedure (CIP) should be done to ensure complete removal.

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 0.1-1% v/v of the packed bed volume.

Elution

It is recommended to use a buffer with an ionic strength equivalent of 0.15 M NaCl or greater to avoid any unwanted ionic interactions between the solute molecule and the resin.

NOTE: *The recommended flow velocity is 10-50 cm/hr (0.15-0.8 ml/min for 11/30 columns, 0.3-1.6 ml/min for 16/600 columns and 0.8 -4.4 ml/min for 26/600 columns. The lower the flow velocity, the better resolution can be attained.*

Maintenance

The resin can be re-used depending upon the application. Cleaning-in-place (CIP) procedure is used to remove strongly bound materials including lipids, endotoxins and denatured proteins from the resin surface.

Cleaning-in-place (CIP)

CIP prevents the bulid-up of contaminants in the packed bed and helps to maintain te column performance.

The frequency of CIP depends upon the specific application and the procedure depend the type of contaminants presents. Below is general guideline for CIP. Optimization is required depending upon the specific use.

Method 1*:* Pass 0.5 M NaOH through resin with reverse flow direction at linear flow velocity of 15-25 cm/hr for 1-2 hrs.

Method 2: Pass 2 bed volumes of 0.1-0.5% detergent in basic or acidic solution at a linear flow velocity of 15-25 cm/hr with reverse flow direction. Remove the residual detergent by washing the column with 5 bed volumes of ethanol or any other alcohol upto 30%v/v or diluted organic solvents.

Method3: Pass 2 bed volumes of dilute organic solvent at linear flow rate of 15-25 cm/hr with reversed flow direction. Organic solvents should be applied in increasing concentration gradients to avoid formation of air bubbles.

After CIP, for all the three methods, the column should be equilibrated with at least 3 CV of equilibration buffer before next run.

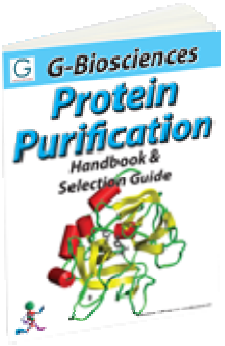
Sanitization: Sanitize the column with at least one bed volume of 0.5 -1.0 M NaOH at flow rate of 10-20 cm/hr. After sanitization equilibrate the column with 3-5 CVs of equilibration buffer.

STORAGE

1. Before storage, pass 2 CV of 20% ethanol at a linear flow velocity ≤30 cm/hr.
2. Attach the leur-thread adaptors and pre-fill both storage syringes with 4-5 ml 20% ethanol. Invert syringes and push out air bubbles.
   NOTE: *Ensure that the remaining fill volume is more than 3 ml.*
3. Keeping the pump running at rate ≤1 ml/min, disconnect the bottom of the column. Screw carefully one storage syringe to the bottom side avoiding trapping of any air bubbles.
4. Stop the pump and disconnect the top column from the chromatograpghy system.
5. Fill the top cavity of column with 20%ethanol and screw the other storage syringe carefully avoiding any trapping of air bubbles.
6. Hold each syringe carefully with one hand and wrap the rubber band to the shoulder of syringe with other hand. Store the column at 4°C for future use.

RELATED PRODUCTS

Download our Sample Preparation and Protein Purification Handbooks.



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

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