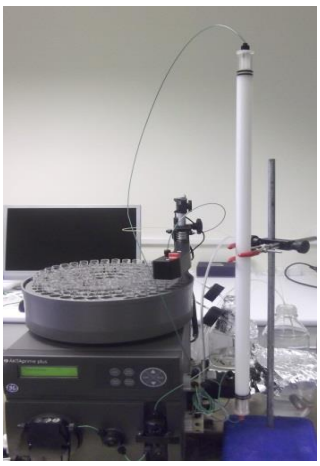
Userguide: ProteoSEC Gel Filtration columns

1. Introduction

Gel Filtration is a proven technique which is widely used for size-based molecular separation. ProteoSEC Gel Filtration resin is specially designed for high resolution and high recovery fractionation of biological molecules based on their molecular weights. Pre-packed ProteoSEC Gel Filtration resin is available in 3 column formats: 11/30, 16/60 and 26/60.

ProteoSEC Gel Filtration resin has a balanced design formulated to offer high recovery and high selectivity according to individual applications. The key benefits are:

- High resolution
- High selectivity
- High recovery
- Highly scalable
- Excellent physical and mechanical stability
- Cost effective



The base matrix is a highly cross-linked polysaccharide composite resin and is very stable in the presence of most aqueous solutions and organic solvents.

ProteoSEC Gel Filtration Resin Selection Guide:

	ProteoSEC 3-70 HR	ProteoSEC 6-600 HR
Separation range	3 kDa – 70 kDa	6 kDa – 600 kDa
Column dimensions	11 mm ID x 30 cm Column Length (28.5 ml CV) 16 mm ID x 60 cm Column Length (120 ml CV) 26 mm ID x 60 cm Column Length (320 ml CV)	
Particle size	20 – 50 µm (35 µm avg.)	20 – 50 µm (35 µm avg.)
Format supplied	Pre-packed 11/30, 16/60 and 26/60 ProteoSEC columns	

ProteoSEC Gel Filtration column specifications:

	ProteoSEC 3-70 HR & ProteoSEC 6-600 HR
Matrix	Highly cross-linked polysaccharide composite of Dextran and Agarose
Particle size (μm)	35 μm (in the range of 20 – 50 μm)
Column body max pressure (*)	6 Bar, 0.6 MPa (3-70 Columns) & 5 Bar, 0.5 MPa (6-600 Columns)
Operating flow velocity	10-50 cm/hour 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 buffers; 1 M acetic acid, 1 M NaOH, 6M guanidine hydrochloride, 8 M urea, 30% isopropanol, 20% ethanol (Concentration of alcohol should not exceed 30% v/v)
Avoid	Oxidizing agents
Storage	0.02% sodium azide or 20% ethanol

* Max resin pressure for all the 3-70 columns is 4 Bar. Max resin pressure for all the 6-600 columns is 3 Bar.

Chromatographic separation and calibration curve the ProteoSEC 16/600 6-600 HR column

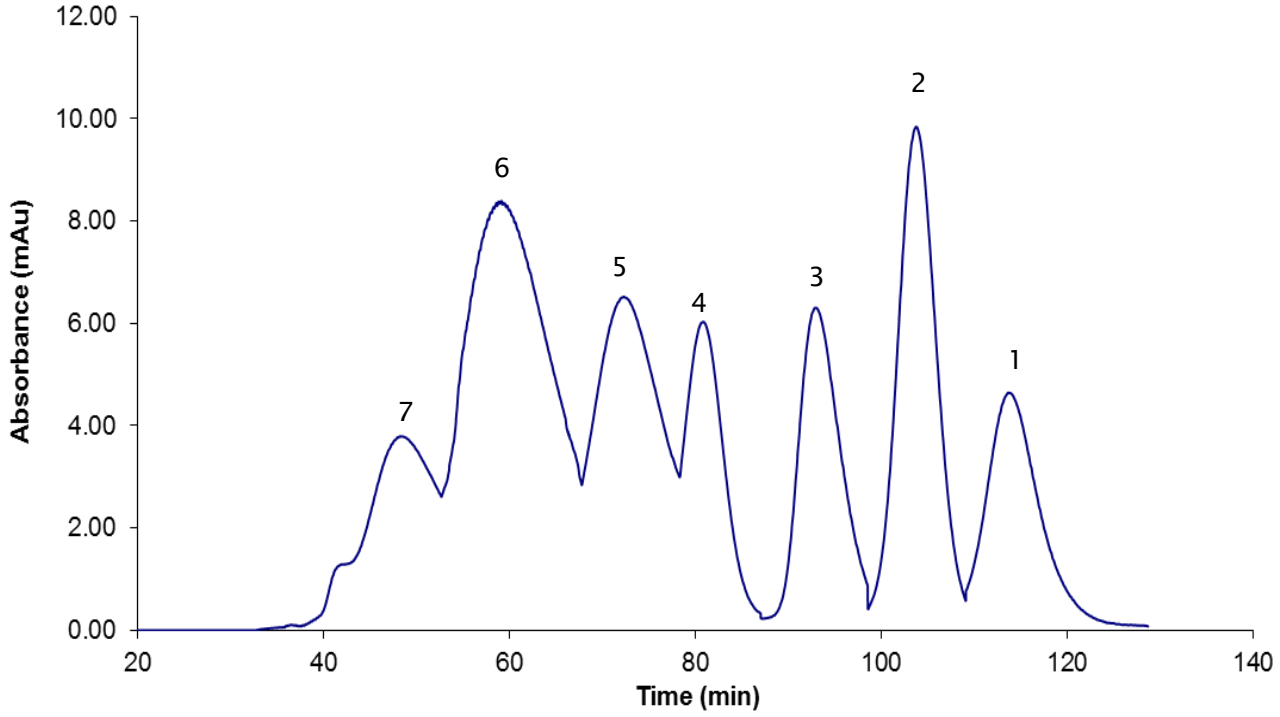


Figure 1: Separation of test substances on a ProteoSEC 16/600 6-600 HR gel filtration column.
Flowrate: 1 ml/min; sample loading 0.5 ml; mobile phase: PBS (phosphate buffered saline); Model proteins 1: Aprotinin (M_r 6,500); 2: Cytochrome c (M_r 12,300); 3: β-Lactoglobulin (M_r 35,000); 4: BSA (M_r 67,000); 5: γ-Globulin IgG (M_r 158,000); 6: Apoferritin (M_r 440,000); 7: Thyroglobulin (M_r 669,000)

Chromatographic separation and calibration curve the ProteoSEC 16/600 3 – 70 HR column

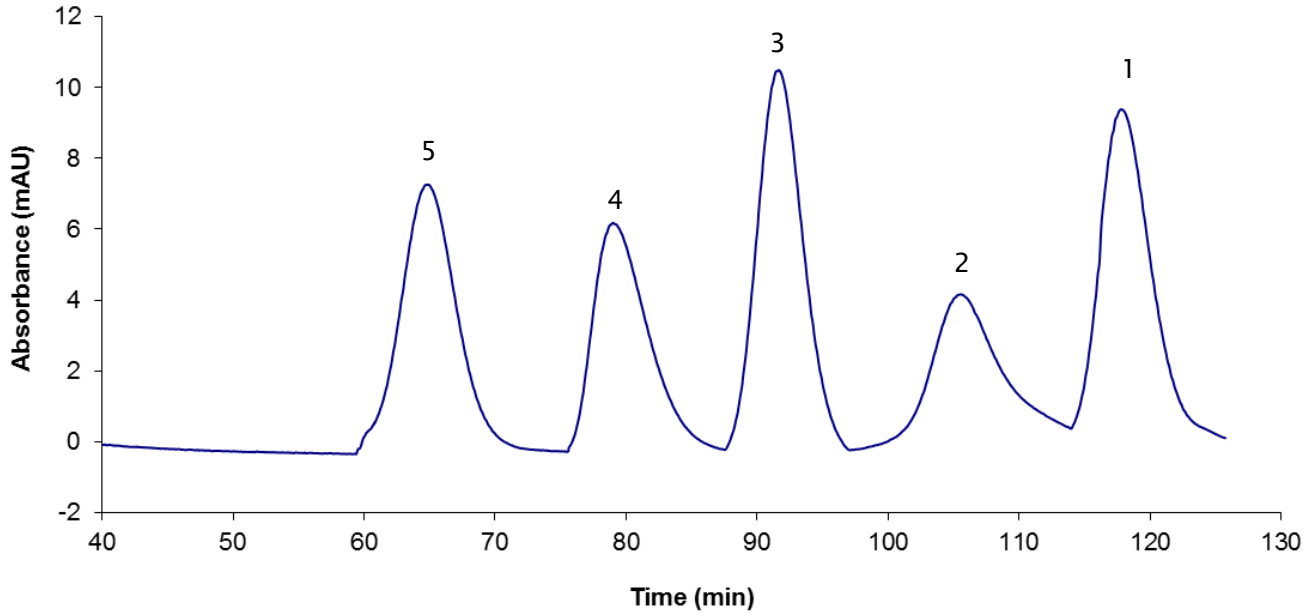


Figure 2: Separation of test substances on a ProteoSEC 16/600 3-70 HR gel filtration column. Flowrate: 1 ml/min; sample loading: 0.5 ml; mobile phase: PBS (phosphate buffered saline); Model proteins 1: Vit B-12 (M_r 1,200); 2: Aprotinin (M_r 6,500); 3: Cytochrome c (M_r 12,300); 4: β-Lactoglobulin (M_r 35,000); 5: BSA (M_r 67,000).

2. Instructions for use

Each packed column is sealed at both ends by mildly pressurised syringes filled with 20% ethanol.

1. Carefully remove all the packaging materials and place the bagged column on a flat surface. Always hold the middle part of the column when handling. ***Never hold the column by the syringe side.*** Cut the plastic bag and slide the column out of the bag.
2. Following the orientation labelling, firmly clamp the column at two points in a vertical position using a suitable support.

Disconnect the top syringe first.

3. Gently unwrap the rubber band from the top syringe.
4. Holding the column body unscrew the 1/16" - tubing adapter from the top of the column. Keep this syringe-tubing set for future use.
5. Connect the chromatography system to the top of the column. The column has a female 1/16” thread connection. If M6 connectors tubing connection is used, a 1/16” male thread – M6 female thread connector is required. It is recommended to have a flow rate ~0.5 ml/min and to fill the thread cavity with liquid before tightening the connection in order to avoid air bubbles.
6. Gently unwrap the rubber band from the bottom syringe.
7. Holding the column body unscrew the 1/16" - tubing adapter from the bottom of the column. Keep this syringe-tubing set for future use.
8. Connect the bottom of the column to the chromatography system.

9. Run the equilibration buffer to displace the storage buffer using a flow rate of up to 1 ml/min for the 16 mm column and 2.5 ml/ml for the 26 mm column. It is recommended to run through at least 2 column volumes of equilibration buffer before sample loading.

3. Method optimization

We recommend the use of a buffer with an ionic strength equivalent to 0.15 M NaCl (or greater) to avoid any undesirable ionic interactions between the target proteins and the resin. In general, the recommended flow velocity is 10 – 50 cm/hour (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 the final resolution. Ideally, the sample volume should be 0.1–1.0% v/v of the packed bed volume.

Before applying a sample, the column should be equilibrated with at least 2 column volumes of buffer until a stable baseline is reached. Re-equilibration between runs is normally not necessary.

In the case that denatured proteins or lipids are not eluted within one column volume, the cleaning-in-place procedure (CIP) should be carried out to ensure their removal.

4. Maintenance

Depending on the individual applications, the resin can be used repeatedly. For re-use, please consider the following instructions.

Cleaning-in-place (CIP)

CIP is a procedure that removes strongly bound materials such as lipids, endotoxins and denatured proteins that remain at the surface of the medium. Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain the column performance.

A specific CIP protocol should be developed for each process according to the type of contaminants present. The frequency of CIP depends on the specific application.

The following methods are for general guidance:

Method 1 Apply a 0.5 M NaOH solution at a linear flow velocity of roughly 15 – 25 cm/hour with a reversed flow direction (to prevent fouling the rest of the resin) for 1 – 2 hours.

Method 2 Apply two bed volumes of 0.1–0.5% detergent in a basic or acidic solution at a linear flow velocity of roughly 15 – 25 cm/hour with reversed flow direction. Residual detergent should be removed by washing the column with five bed volumes of alcohols (eg. ethanol) up to 30% v/v or other diluted organic solvents.

Method 3 Apply two bed volumes of a dilute organic solvent (eg. 30% isopropanol) at a linear flow velocity of roughly 15 – 25 cm/hour with reversed flow direction. To avoid the formation of air bubbles, organic solvents should be applied in increasing concentration gradients.

For all methods, after the CIP step, the column should be equilibrated with at least 3 column volumes of buffer before the next run.

Sanitization Sanitize the column using at least 1 bed volume of 0.5-1.0 M NaOH at a flow flow velocity of 10- 20 cm/hour. Following sanitization, the column should be re-equilibrated with 3-5 CVs of buffer.

5. Storage instruction

1. Run through 2 column volumes of 20% ethanol at a linear flow velocity of ≤30 cm/hr.
2. Attach the luer-thread adapters and pre-fill both storage syringes with approx. 4 to 5 ml 20% ethanol. Invert each syringe and push any air bubbles out. Make sure the fill volume remaining is over 3 ml.
3. Keep the pump running at a flow rate ≤1 mL/min. Disconnect the bottom of the column. Screw one storage syringe to the bottom side making sure that no air bubbles are trapped.
4. Stop the pump. Disconnect the top of the column from the chromatography system.
5. Fill the top cavity of the column with 20% ethanol using a pipette.
6. Screw the other storage syringe into the top of the column ensuring that no air bubbles are trapped.
7. Carefully hold each syringe with one hand and wrap the rubber band with the other hand to the shoulder of the syringe.
8. Place the column at 2 – 8 °C for long term storage.

6. Ordering information

Product Name	Product Description	Units	Order Code
ProteoSEC 11/30 3-70 HR SEC Column	ProteoSEC SEC column (11 mm ID; 30 cm length, 3-70 kDa HR resin) 28.5 ml bed volume for max 250-500 µl sample volume	1	SEC-11/30-3-70
ProteoSEC 16/60 3-70 HR SEC Column	ProteoSEC SEC column (16 mm ID; 60 cm length, 3-70 kDa HR resin) 120 ml bed volume for max 1-5 ml sample volume	1	SEC-16/60-3-70
ProteoSEC 26/60 3-70 HR SEC Column	ProteoSEC SEC column (26 mm ID; 60 cm length, 3-70 kDa HR resin) 320 ml bed volume for max 3-10 ml sample volume	1	SEC-26/60-3-70
ProteoSEC 11/30 6-600 HR SEC Column	ProteoSEC SEC column (11 mm ID; 30 cm length, 6-600 kDa HR resin) 28.5 ml bed volume for max 250-500 µl sample volume	1	SEC-11/30-6-600
ProteoSEC 16/60 6-600 HR SEC Column	ProteoSEC SEC column (16 mm ID; 60 cm length, 6-600 kDa HR resin) 120 ml bed volume for max 1-5 ml sample volume	1	SEC-16/60-6-600
ProteoSEC 26/60 6-600 HR SEC Column	ProteoSEC SEC column (26 mm ID; 60 cm length, 6-600 kDa HR resin) 320 ml bed volume for max 3-10 ml sample volume	1	SEC-26/60-6-600

Last update: 08 April 2016

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