anatrace

ProteoSEC Gel Filtration Columns Userguide

Product numbers: SEC-16/60-3-70
SEC-26/60-3-70
SEC-16/60-6-600
SEC-26/60-6-600

DETERGENTS | LIPIDS | CUSTOMS | HIGHER STANDARDS

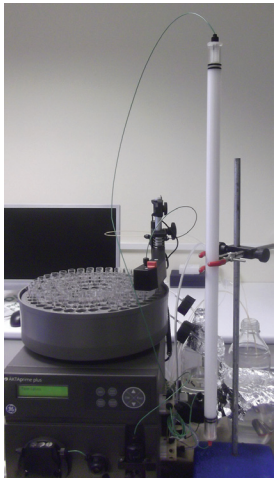
DETERGENTS | LIPIDS | CUSTOMS | HIGHER STANDARDS

434 W. Dussel Drive | Maumee, OH 43537 | **P:** 800-252-1280 | 419-740-6600 | **F:** 419-740-6630
E: customerservice@anatrace.com | **W:** www.anatrace.com

Table of Contents:

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 2 column formats: 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:	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 16/60 and 26/60 ProteoSEC columns	

ProteoSEC Gel Filtration Column Specifications:

	ProteoSEC 3-70 HR and 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) and 5 Bar, 0.5 MPa (6-600 columns)	
Operating flow velocity:	10-50 cm/hour	0.3–1.6 ml/minute (16/60 columns) 0.8–4.4 ml/minute (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, 6 M 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	

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

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

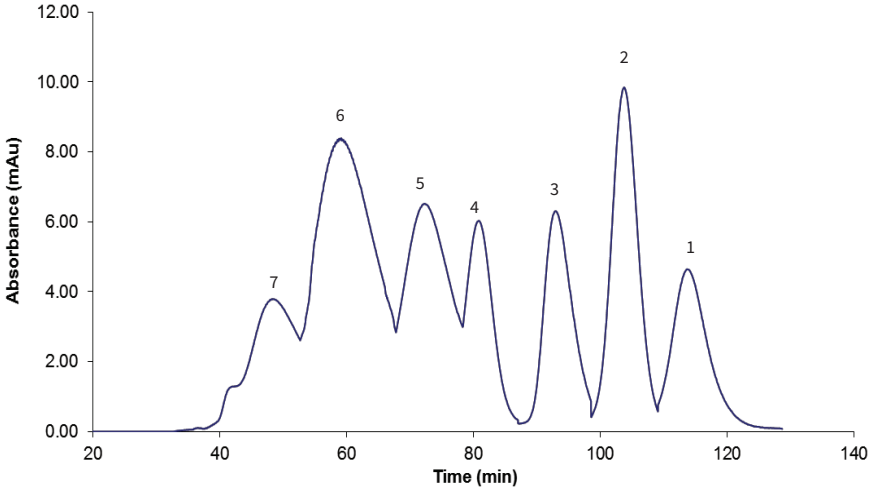


Figure 1: Separation of test substances on a ProteoSEC 16/600 6-600 HR Gel Filtration Column.

Flowrate: 1 ml/minute; 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

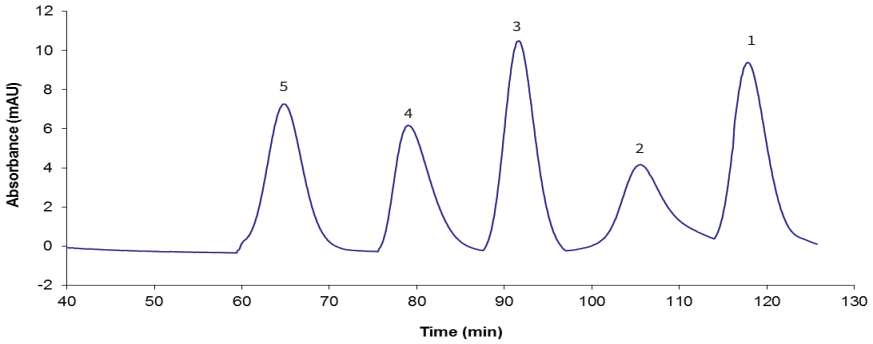


Figure 2: Separation of test substances on a ProteoSEC 16/600 3-70 HR Gel Filtration Column.

Flowrate: 1 ml/minute; 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).

Instructions for Use

Each packed column is sealed at both ends by mildly pressurized 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. Hold the syringe body firmly, then twist to unscrew the luer-thread adaptor from the column top. Keep this syringe for future use.
5. Connect the column top to the chromatography system. It is recommended to have a flow rate ~0.5 ml/minute 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. Disconnect the bottom syringe. Hold the syringe body, then twist to unscrew the luer-thread adaptor from the column. Keep this syringe 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/minute. It is recommended to run through at least 2 column volumes of equilibration buffer before sample loading.

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 (0.3–1.6 ml/minute for 16/600 columns and 0.8–4.4 ml/minute 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.

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 (*e.g.* ethanol) up to 30% v/v or other diluted organic solvents.

Method 3:
Apply two bed volumes of a dilute organic solvent (*e.g.* 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 velocity of 10–20 cm/hour. Following sanitization, the column should be re-equilibrated with 3–5 bed volumes of buffer.

Storage Instructions

1. Run through 2 column volumes of 20% ethanol at a linear flow velocity of ≤30 cm/hour.
2. Attach the luer-thread adapters and pre-fill the storage syringes with approx. 4 to 5 ml 20% ethanol. Invert the 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/minute. Disconnect the bottom of the column. Screw the 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 storage syringe into the top of the column making sure 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.

Ordering Information

Product Name	Product Description	Units	Product No.
ProteoSEC Size Exclusion Column 16/60 3-70 HR SEC	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 Size Exclusion Column 26/60 3-70 HR SEC	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 Size Exclusion Column 16/60 6-600 HR SEC	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 Size Exclusion Column 26/60 6-600 HR SEC	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

To order:

Telephone: Monday-Friday 8:00 AM–5:00 PM (EST)
Toll free in the U.S. 1-800-252-1280
Or call 419-740-6600

Mail: Anatrace Products, LLC
434 West Dussel Drive
Maumee, OH 43537

Fax: 419-740-6630

Email: customerservice@anatrace.com

Web: www.anatrace.com

For research use only.
Anatrace is a registered trademark of Anatrace Products, LLC.

rev 11/15