ENrich™ SEC 70
ENrich™ SEC 650
High-Resolution Size Exclusion Columns

Instruction Manual

Catalog numbers

780-1070
780-1650

Please read these instructions before you use ENrich SEC high-resolution size exclusion media. If you have any questions or comments regarding these instructions, please contact your Bio-Rad Laboratories representative.

BIO-RAD

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Section 1: Characteristics of the ENrich™ Size Exclusion Columns

1.1 Introduction

ENrich prepacked columns for size exclusion chromatography are designed for rapid and reproducible high-resolution separation of biomolecules. Each column contains a unique, high-performance size exclusion media based on spherical polymer beads modified to provide quick, differential size–based diffusion.

1.2 The ENrich SEC Separation Media

ENrich SEC media is available with different exclusion limits and separation ranges. The prepacked columns contain 10 µm beads, which produce excellent resolution of biomolecules at low backpressures. SEC 70 is ideal for separating biomolecules up to 70,000 Da and SEC 650 is used for larger biomolecules up to 650,000 Da.

The 10 µm particle size and narrow particle size distribution provide excellent resolution of biomolecules at high flow rates and with relatively low backpressures. The hydrophilic ENrich media demonstrates extremely low nonspecific binding of biomolecules accompanied by high recovery of biological activity.

Stability

The columns are stable over the pH range 2–12, allowing easy cleaning and regeneration.

The ENrich support is compatible with aqueous solutions of 6 M guanidine-HCl and 8 M urea. Detergents and organic solvents such as methanol, ethanol, and isopropanol may also be used. In some cases, alcohols and stability agents such as glycerol may increase the column backpressure. Care should be taken to maintain a flow rate at which the backpressure is below the maximum operating pressure.

Table 1. ENrich SEC media and column characteristics.

	SEC 70	SEC 650
Linear separation range, Da	500–70, 000	500–650, 000
Column dimensions, diameter x height, mm	10 x 300	10 x 300
Column volume, ml	24	24
Nominal particle size, µm	10 ± 2	10 ± 2
Recommended flow rates, ml/min*	0.5–1.0	0.75–1.25
Maximum recommended flow rates, ml/min*	1.5	2.0
Maximum operating pressure	600 psi, 4.1 MPa, 41 bar	600 psi, 4.1 MPa, 41 bar
Recommended sample volume, µl	<250	<250
Efficiency, plates/m	>20,000	>20,000
Working pH range	2–12	2–12
Operating temperatures, °C	4–40	4–40

* At room temperature. Viscosity may increase at lower temperatures, which will reduce the recommended flow rates.

1.3 Connection to the NGC™ and Other Chromatography Systems

The ENrich columns are fitted with 10-32 type female fittings on either end. Standard 10-32 fittings can be used to plumb the column to a Bio-Rad® NGC™ chromatography system or another vendor's systems. Adaptors are available for connection to systems that use 1/4-28 fittings (catalog # 750-0564).

Section 2: Use of the ENrich Columns

2.1 Preparation for Initial Use

The column is supplied in 20% ethanol. To remove the storage buffer, pump deionized, filtered water at a flow rate of 0.5 ml/min or less for 30 ml, or just over one column volume (CV). The backpressure will decrease as the ethanol is washed from the column. After this water step you may equilibrate your column as described below.
Warning: Do not exceed a maximum pressure of 600 psi (4.1 MPa, 41 bar).

2.2 Equilibrating and Running the Column

1. Always use filtered (0.22–0.45 µm filter) and degassed buffers. This will prolong the life of your column. To avoid bacterial growth, contamination, and poor column performance, use only freshly prepared buffers.
2. Residual ionic charges on the ENrich column are negligible. However, a running buffer with ionic strength of at least 100 mM NaCl is recommended. Phosphate buffered saline (PBS) is an example of a commonly used buffer appropriate for ENrich SEC media.
3. The buffer should contain any cofactors or protease inhibitors previously identified as being essential to maintain enzyme activity.
4. Equilibrate the column with at least 2 CV (~50 ml) of the buffer.
5. Calibrating the column with a gel filtration standard (catalog #151-1901) is an important step for molecular weight determination and for tracking column performance.

2.3 Sample considerations

1. Always filter (0.22–0.45 µm filter) and/or centrifuge your sample to remove any particulates.
2. In size exclusion chromatography, mass loading is less critical than the volume of the sample. As a general rule, to obtain maximum performance, the sample volume should not exceed 1% of the bed volume (that is, ~0.25 ml for a 24 ml CV) and the sample concentration should not exceed 20 mg protein/ml. Larger volumes can be applied, but resolution may decrease.

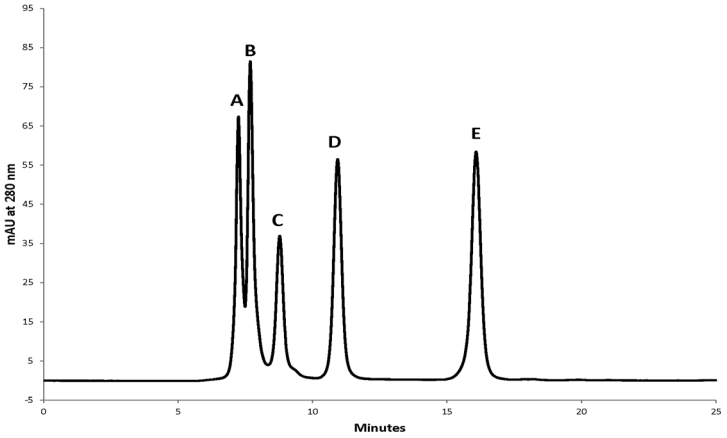
2.4 Sample Elution

All species within the sample should elute within 1 full CV. The flow rate for optimal resolution depends on sample composition and purity. In general, lower flow rates provide greater performance. Optimal flow rates for both ENrich SEC 70 and ENrich SEC 650 columns are 0.5–1.25 ml/min (see Table 1).

2.5 Column Calibration

Use the Bio-Rad size exclusion standards (catalog #151-1901). One vial contains 18 mg of a lyophilized mixture of thyroglobulin (Mr 670,000), bovine γ-globulin (Mr 158,000), chicken ovalbumin (Mr 44,000), equine myoglobin (Mr 17,000), and vitamin B12 (Mr 1,350).

ENrich SEC 70 media



ENrich SEC 650 media

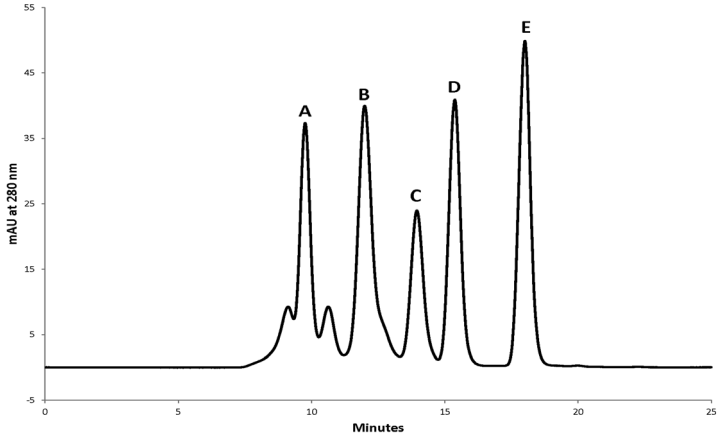


Figure 1. Typical chromatograms for ENrich SEC media. Detection at 280 nm. Absorbance at 280 nm may be greater depending on sample concentration. Time may change depending on flow rate. **A**. thyroglobin; **B**. IgG; **C**. ovalbumin; **D**. myoglobin; **E**. vitamin B12.

Section 3: Care of the ENrich SEC Column

3.1 Regular Maintenance

To maintain performance, the column should be stored in 20% ethanol, as described below, for storage periods longer than 2 days. Always run at least one CV of water between 20% ethanol and running buffers.

If the column is exposed to air or if air bubbles are trapped in the column.

1. Wash the column in the forward direction with 30 ml of water at 0.25–0.5 ml/min.
2. Wash the column with 30 ml of 20% ethanol at 0.25–0.5 ml/min. Note that the backpressure will increase in ethanol. Do not exceed 600 psi (4.1 MPa).
3. Wash the column with 30 ml of deionized water at the usual operating flow rate as recommended in Table 1.
4. Re-equilibrate the column with the desired buffer.

If the column begins to show abnormally high backpressure.

If the backpressure increases abnormally, it is likely that the column needs to be cleaned and/or the frit at the top of the bed is clogged. First, clean the column using the method described in section 3.3. If this is not sufficient, replace the top frit as described in section 3.4.

3.2 Bed Height Adjustment

Under certain conditions of buffer composition, high flow rates, or long-term use, the resin bed may compress, creating a void between the frit and the top of the bed. Normally, the void can be eliminated by turning the adjusting nut clockwise until the frit just touches the top of the bed. If the bed compresses at high flow rates, stop the pump and loosen the top fitting, then use the adjusting nut to remove the void, retighten the top fitting, and resume pumping buffer. If the bed has compressed from long-term use, replace the top frit as a precaution.

3.3 Column Cleaning

Careful preparation (especially filtration) of the sample and the buffers will maintain the column's performance and extend its lifetime. Normally, washing with a few CV of running buffer with an ionic strength of at least 100 mM NaCl will remove most contaminants. However, if there is a decrease in column performance (that is, increasing backpressures or a drop in resolution), then steps 1 and 2 of the cleaning protocol described below should be used.

Always reverse the flow during this procedure so any substances that may be trapped at the top of the column are quickly removed. During this operation do not exceed more than 50% of the recommended maximum flow rate (see Table 1). Ensure that the backpressure stays below the maximum operating pressure.

1. Wash with 2 x 1 ml injections of 2.0 M NaCl followed by 1 CV of buffer.
2. Wash with 2 x 1 ml injections of 1.0 M NaOH followed by 2 CV of buffer.

If the column performance has still not returned, the additional steps below can be followed:

3. Wash with 2 x 1 ml injections of 50% acetic acid followed by 2 CV of buffer.
4. If lipid contamination is suspected, first wash with at least 1 CV of deionized water. Then wash with 1 CV of 70% ethanol followed by 2 CV of water. The flow rate may need to be slowed significantly during the 70% ethanol wash.

Before use, re-equilibrate with at least 2 CV of equilibration buffer.

3.4 Frit Replacement

The top frit may need to be replaced after extensive column use or if increasing backpressures are noticed. Always try to clean the column in the reverse direction (as described in section 3.3) before replacing the frit. A frit kit containing a frit removal tool, 2 O-rings, and 2 frits is available.

Figure 2 shows a column diagram to assist in the replacement of the top frit.

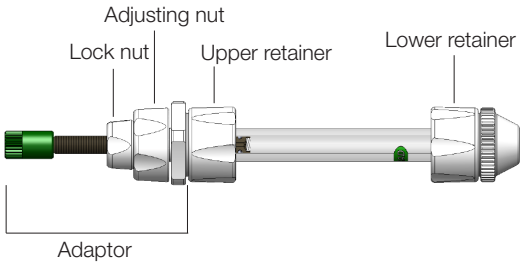


Fig. 2. Column diagram.

1. Start a slow flow rate of buffer (0.5 ml/min or less) through the column.
2. Remove the lower end tubing and fitting from the column. Firmly hold the bottom of the column over a sink or container.
3. Loosen the lock nut by turning it clockwise.
4. Raise the adaptor a few millimeters by slowly by turning the adjusting nut counterclockwise.
5. Unscrew the upper retainer. Let it rest on the bottom retainer.
6. Slowly pull out the upper adaptor from the glass column. Allow the buffer flow to maintain the integrity of the top of the bed.
7. Stop the pump. Plug the bottom of the column. Set the column upright in a beaker. Remove the tubing and fitting from the top of the adaptor.
8. Remove the frit from the adaptor by hooking one end of the frit removal tool/tweezer into the frit in a sideways motion with slight downward pressure.
9. Push the new frit into the end of the adaptor.
10. Replace the O-rings if they appear worn or torn. If the O-rings are replaced, wet them with buffer before the next step.
11. Add a few drops of buffer to the top of the resin bed. Insert the adaptor and push it down to the bed. Some buffer should flow out the top of the column.
12. Screw on the upper retainer.
13. Lower the adaptor to the top of the bed by turning the adjusting nut clockwise.
14. Tighten the lock nut by turning it counterclockwise.

3.5 Storage Conditions

To prevent bacterial growth in the column, it must be stored correctly. For short-term storage (up to 2 days), store it in freshly prepared buffer. For long-term storage, first wash the column with 30 ml of deionized water and then with a storage solution of 20% ethanol. Use a flow rate of 0.5 ml/min or less. Ensure that the backpressure stays below the maximum operating pressure. Always store the column with plugs at each end. Store the column in a safe place at room temperature or 4°C. Never allow the column to freeze.

If, during storage or shipment the column might be exposed to temperatures above 35°C, briefly (3 min under vacuum) degas the 20% ethanol storage solution before use.

Section 4: Ordering Information

Catalog #	Description
780-1070	**ENrich SEC 70 10 x 300 Column**, 10 x 300 mm
780-1650	**ENrich SEC 650 10 x 300 Column**, 10 x 300 mm
780-0093	**ENrich 10 Frit Kit**, includes 2 frits, 1 frit remover, 2 O-rings
151-1901	**Gel Filtration Standard**, 6 vials

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