
ENrich™ Q **ENrich™ S** **High-Resolution Ion** **Exchange Columns**

Instruction Manual

Catalog numbers

780-0001

780-0003

780-0021

780-0023

Please read these instructions before you use ENrich Q and S high-resolution ion exchange media. If you have any questions or comments regarding these instructions, please contact your Bio-Rad Laboratories representative.



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Section 1: Characteristics of the ENrich Ion Exchange Columns

1.1 Introduction

ENrich prepacked ion exchange columns meet the needs of the biochromatographer for rapid and reproducible high-resolution separations of proteins and other biomolecules, including peptides and polynucleotides. Two column sizes (bed volumes of 1 and 8 ml) provide predictable scale-up of high-resolution separations without sacrificing capacity.

1.2 The ENrich Separation Media

Each column contains a spherical, rigid, and highly porous polymeric support derivatized with the strongly basic $-N^+(CH_3)_3$ quaternary ammonium group or the strongly acidic $-SO_3^-$ group. The 10 μm particle-size and narrow particle size distribution provide excellent resolution of biomolecules at high flow rates and with low backpressures. The hydrophilic ENrich media demonstrate extremely low non-specific binding of biomolecules accompanied by high recovery of biological activity.

Stability

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

The ENrich support is compatible with aqueous solutions of 6 M guanidine-HCl and 8 M urea. Nonionic detergents and organic solvents such as methanol, ethanol, and isopropanol may also be used. Ionic detergents can be used only if the detergents have the same charge as the support (that is, sodium dodecyl sulfate (SDS), a negatively charged detergent, should not be used with a positively charged anionic exchanger like ENrich Q).

Table 1. Column characteristics.

	Q		S	
Column dimensions (diameter x height, mm)	5 x 50	10 x 100	5 x 50	10 x 100
Column volume, ml	1	8	1	8
Maximum protein capacity, mg				
Bovine serum albumin	130	1000	–	–
Human IgG	–	–	120	940
Average particle size, μm	10	10	10	10
Maximum operating pressure, psi	500	500	500	500
Recommended flow rate, ml/min*	1	4	1	4

* 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 Bio-Rad's NGC chromatography system or other vendors' 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 columns are supplied in a storage solvent of 20% ethanol in water. Prior to initial use and after extended storage periods, each column should be conditioned as described in steps 1–4. Always use HPLC-grade reagents and be sure to filter and degas solvents. During these four steps do not exceed more than 50% of the recommended flow rates (see Table 1).

1. Wash with 5 column volumes of water.
2. Wash with 5 column volumes of low ionic strength equilibration buffer (such as 20 mM buffer salt).
3. Wash with 5 column volumes of high ionic strength limit buffer (such as 1.0 M NaCl).
4. Wash with 5 column volumes of low ionic strength equilibration buffer.

The column may now be further equilibrated in the start buffer at the desired flow rate.

2.2 Buffer Selection

Table 2 lists commonly used buffers for ion exchange chromatography. The buffers are specific to the type of ion exchange. Therefore, it is important not to use anionic buffers with the ENrich Q, which would interact with the anionic exchange group on the support.

The choice of whether to use an anion or cation exchanger is determined mainly by the isoelectric point (pI) and the relationship between pH and the activity/stability of the protein(s) of interest. When the type of ion exchanger is determined, the pH-activity relationship also determines the choice of buffer. As a general rule, the chosen buffer should be used within ± 1.0 pH unit of its pKa value. This permits use of the lowest possible buffer concentration while maintaining maximum buffering capacity. We recommend a minimum buffer concentration of 20 mM.

Table 2. Buffers for ion exchange chromatography.**Anion Exchange — ENrich Q**

pH Range	Buffer	MW	pKa @ 25°C	Counter-Ion	pKa/°C
5.0–6.0	Piperazine	86.1	5.7	Cl ⁻ /HCOO ⁻	-0.015
5.0–6.0	L-histidine	155.2	6.15	Cl ⁻	–
5.8–7.2	Bis-Tris	209.2	6.5	Cl ⁻	-0.017
6.4–7.3	Bis-Tris propane	282.3	6.8,9	Cl ⁻	–
7.3–8.3	Triethanolamine	149.2	7.8	Cl ⁻ /CH ₃ COO ⁻	-0.02
7.6–8.6	Tris	121.1	8.1	Cl ⁻	-0.031
8.4–8.8	Diethanolamine	105.1	8.9	Cl ⁻	-0.025
9.0–9.9	Ethanolamine	61.1	9.5	Cl ⁻	-0.029
9.8–10.3	1,3-diamino-propane	74.1	10.5	Cl ⁻	-0.023

Cation Exchange — ENrich S

pH Range	Buffer	MW	pKa @ 25°C	Counter-Ion	pKa/°C
3.6–4.3	Lactic acid	90.1	3.8	Na ⁺	–
4.2–5.2	Citric acid	192.1	3.1	Na ⁺	–
5.5–6.7	MES	195.2	6.1	Na ⁺	-0.011
6.1–7.5	PIPES	302.4	6.8	Na ⁺	-0.009
6.5–7.9	MOPS	209.3	7.2	Na ⁺	-0.006
6.7–7.6	Phosphate	120/142	7.2	Na ⁺	-0.003
6.8–8.2	TES	229.2	7.4	Na ⁺	-0.002
6.8–8.2	HEPS	238.3	7.5	Na ⁺	-0.014
7.4–8.8	Tricine	179.2	8.1	Na ⁺	-0.021

Always use buffer components of the highest purity available, as UV-absorbing impurities may cause baseline disturbances and interfere with the detection of protein peaks. Always filter and degas buffers.

2.3 Sample Preparation

Proper adjustment of the sample pH and ionic strength is critical for consistent and reproducible chromatography. For best results, the sample should be exchanged into the start buffer or diluted to the start buffer's concentration. Buffer exchange can be performed using Bio-Spin® 6 and Micro Bio-Spin™ 6 columns, Econo-Pac® 10DG desalting columns, Bio-Gel® P-6DG gel, or the Bio-Scale™ Mini desalting cartridges with Bio-Gel P-6. The choice of product depends on sample volume. Centrifuge or filter the sample (0.2–0.45 µm filter) to remove particulates. Application of turbid or lipid-containing samples may reduce the column lifetime.

Sample Load

The maximum sample load for each column is shown in Table 1. This amount may vary somewhat depending on the actual sample composition. We do not recommend overloading the column as both resolution and column lifetime will decrease. For larger loads, perform several chromatographic runs with a reduced load. Ideally, samples should be bound in a concentrated zone at the top of the column. Higher sample loads produce a broad application zone in which components with less charge are displaced by more highly charged components. This may result in a shift of certain peaks to an earlier elution position in the gradient. The recommended sample load is approximately 20% of the maximum.

2.4 Elution Conditions

Separations by ion exchange are typically accomplished by increasing the salt concentration of the eluent either as a step or as a continuous gradient. Sodium chloride (NaCl) and potassium chloride (KCl) are the most common elution salts. For many separations, varying the pH of the elution buffer in addition to its salt concentration may be advantageous.

Gradient Volumes and Salt Concentrations

As a starting point for developing a separation, we recommend using the ENrich column with a simple linear gradient over 15 column volumes (15 ml).

Suggested protocol for ENrich 5 x 50 ion exchange columns:

- Use a flow rate of 1.0 ml/min. Equilibrate the column with 5 column volumes (5 ml) of equilibration buffer. Then apply the sample. Following sample application, wash unbound proteins from the column with 3 column volumes (3 ml) of equilibration buffer A. For elution, use a gradient volume of 15 column volumes (15 ml) to an NaCl concentration of 0.5 M.
- Follow this segment of the gradient by stepping the salt concentration to 1.0 M (100% B) and then hold at 1.0 M for 3 ml before re-equilibrating the column with 3 ml of start buffer A. This gradient is shown schematically in Figure 1. When an initial separation has been performed and the elution position of the protein of interest determined, the gradient composition and volume are adjusted to achieve maximum resolution. Normally, a gradient volume of 10 to 20 ml per ml of column bed volume is sufficient.
- The slope of the gradient will affect resolution. A steep gradient will result in relatively small peak volumes but short peak-to-peak distances. A shallower gradient normally gives greater resolution but peak volumes are larger.

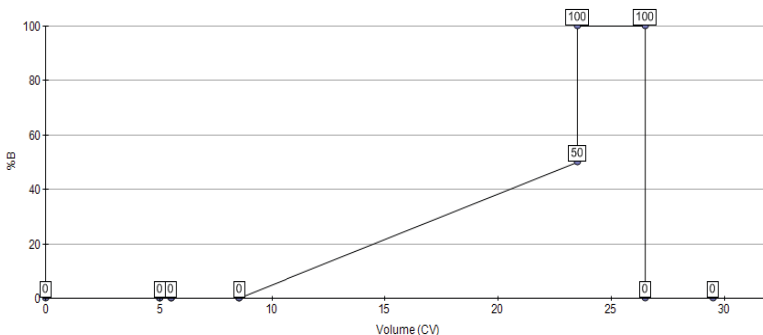


Fig. 1. Schematic gradient for separation on an ENrich IEX column. Volume, column volume.

Section 3: Care of the ENrich Ion Exchange Column

3.1 Column Cleaning

Careful preparation (especially filtration) of the sample and the buffers will maintain the column performance and lifetime. Normally, washing with 1.0 M NaCl or KCl will remove most bound components. However, if there is a significant decrease in column performance (increasing backpressures or a significant drop in resolution), then a more extensive cleaning protocol such as that described in steps 1–6 should be used. **Always reverse the flow during this procedure so tightly bound substances at the top of the column are removed quickly.**

During this operation do not exceed more than **50%** of the recommended flowrates (see Table 1).

1. Wash with 2 column volumes of 2.0 M NaCl or KCl followed by 3 column volumes of water.
2. Wash with two 100 μ l injections of 1.0 M NaOH followed by 3 column volumes of water.
3. Wash with two 100 μ l injections of 50% acetic acid followed by 3 column volumes of water.
4. If lipid contamination is suspected, wash with 1 column volume of 20% ethanol followed by 3 column volumes of water.
5. Wash with 2 column volumes of 2.0 M NaCl or KCl, or the salt containing the desired counter-ion.
6. Wash with 3 column volumes of equilibration buffer.

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 (Figure 2). 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 use, the top frit should be replaced as a precaution.

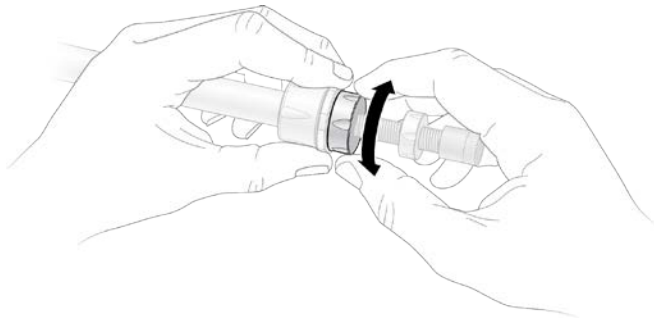


Fig. 2. Turning the adjusting nut raises or lowers the adaptor.

3.3 Frit Replacement

The top frit may need to be replaced after extensive column use or if increasing backpressures are noticed. Always try cleaning the column in the reverse direction (as described in Section 3.1) before replacing the frit. A frit kit is available which contains a frit removal tool, 2 O-rings and 2 frits.

Figure 3 shows a column diagram to assist in the replacement of the top frit.

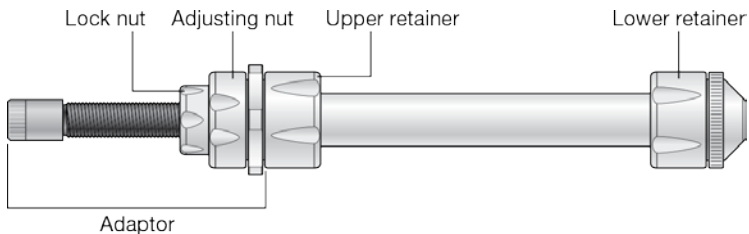


Fig. 3. Column diagram.

1. Start buffer through the column at a slow flow rate (0.5 ml/min or less).
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 turning it clockwise (Figure 4).

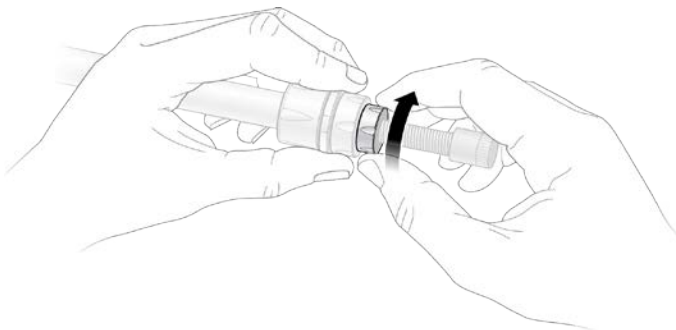


Fig. 4. Loosen the lock nut by turning it clockwise.

4. Raise the adaptor a few millimeters by slowly by turning the adjusting nut counterclockwise (Figure 5).

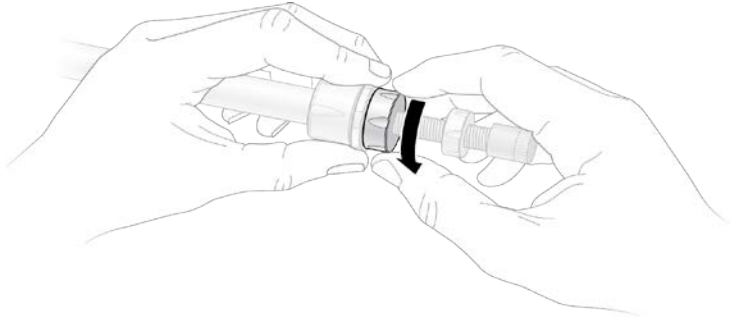


Fig. 5. Raise the adaptor by turning the adjusting nut counterclockwise.

5. Unscrew the upper retainer. Let it rest on the bottom retainer (Figure 6).

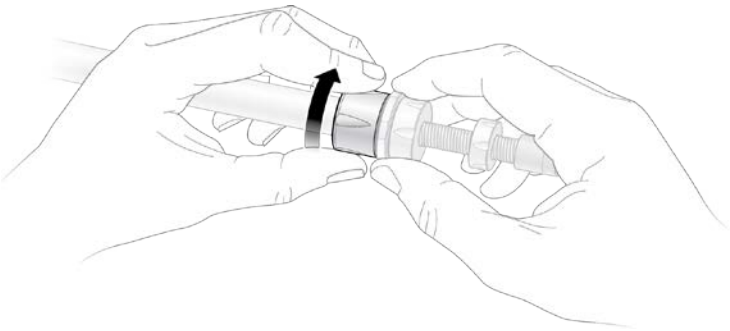


Fig. 6. Remove the retainer.

6. Slowly pull out the upper adaptor from the glass column. Allow the buffer flow to continue in order 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 of the top of the column.
12. Screw on the upper retainer (Figure 7).

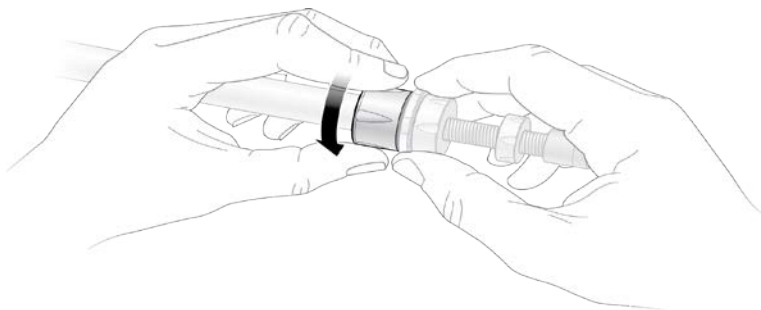


Fig. 7. Reattach and hand-tighten the retainer.

13. Lower the adaptor to the top of the bed by turning the adjusting nut clockwise (Figure 8).

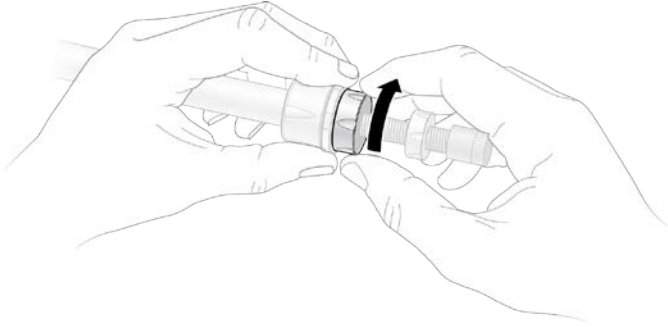


Fig. 8. Lower the adaptor by turning the adjusting nut clockwise.

14. Tighten the lock nut by turning it counterclockwise (Figure 9).

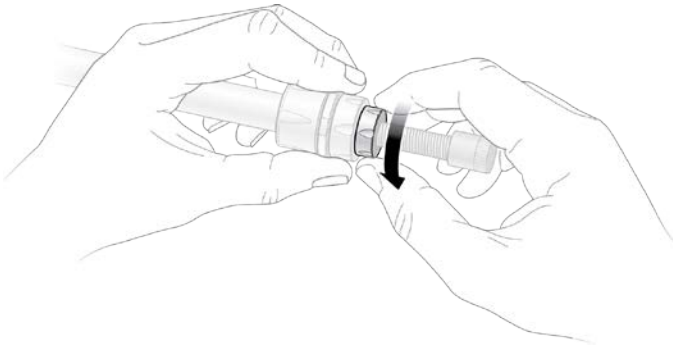


Fig. 9. Hand-tighten the lock nut by turning it counterclockwise.

3.4 Storage Conditions

Prior to long-term storage, the column should be cleaned as previously described and then washed with 3 column volumes of 20% ethanol at a flow rate that is 50% of the recommended flow rate (see Table 1). This will prevent microbial growth. Store the column in a safe place at room temperature or 4°C. Never allow the column to freeze.

Section 4: Product Information

Catalog Number	Product Description
780-0001	ENrich Q 5 x 50 Column
780-0003	ENrich Q 10 x 100 Column
780-0021	ENrich S 5 x 50 Column
780-0023	ENrich S 10 x 100 Column
780-0091	ENrich 5 Frit Kit , includes 2 frits, 1 frit remover, 2 O-rings
780-0093	ENrich 10 Frit Kit , includes 2 frits, 1 frit remover, 2 O-rings
780-0008	1/16" 10-32 Male Fittings , 2/pk

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