UNO™ Q&S Polishing Column for Continuous Bed Ion Exchange Chromatography

Instruction Manual

Catalog Numbers
720-0009
720-0029

BIO-RAD

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UNO Q & S Polishing Ion Exchange Column

Introduction

UNO prepacked ion-exchange columns are designed to meet the needs of the bio-chromatographer for rapid and reproducible high-resolution separations of biomolecules including proteins, peptides and polynucleotides. The UNO Polishing column is designed specifically as a late-stage purification tool to obtain the highest resolution and recovery from a small sample load (up to 2 mg). The small column volume means that peak elution volumes are usually 0.2 ml or less. This presents an unique tool to purify and concentrate dilute samples in one step. However, the polishing column may be used as an economical scouting tool to investigate the conditions for purifying a protein before using a standard UNO column. The polishing columns are constructed with biocompatible PEEK and polyethylene frits to maintain biological activity to insure biological activity of the sample.

The UNO Separation Medium

Each column contains a "Continuous Bed" support derivatized with either strongly basic ($-N^+(CH_3)_3$) or strongly acidic $-SO_3^-$ sulfonic groups. The novel support produces excellent resolution of biomolecules at high flow rates and with low back-pressures. The hydrophilic support demonstrates extremely low non-specific binding of biomolecules accompanied by high recovery of biological activity.

Stability of the UNO Support

The columns are stable over the pH range 2–12, allowing for easy cleaning and regeneration. The support is compatible with aqueous solutions of guanidine-HCl and urea. Detergents and organic solvents such as 100% methanol, 100% ethanol, 100% isopropanol, and 30% acetonitrile may also be used.

Connection to Chromatography Systems

Each UNO column is supplied with a fittings kit containing two pieces of 0.02 inch ID (1/16 inch OD) Tefzel tubing and two 1/4 x 28 flangeless fittings. Assemble one 1/4 x 28 fitting onto one end of the 0.02" tubing and insert each fitting into the top and bottom of the column. For connection to your chromatography system, attach the appropriate fitting onto the bare end of the Tefzel tubing.

Note: Bio-Rad Laboratories does not recommend nor warranty the use of the UNO columns with solvent delivery systems containing stainless steel parts when used with corrosive eluents containing *e.g.* halide salts. We recommend the

use of inert, biocompatible (ceramic, PEEK, titanium) solvent delivery systems for maximum column life and recovery of sample biological activity.

Table 1. Column Characteristics

	Q Polishing Column	S Polishing Column
Column volume (ml)	0.16	0.16
Recommended max. protein loading (mg)	2	2
Recommended flow-rates (ml/min)	0.1 to 1	0.1 to 1
Column Dimensions (mm)	4.6 x 10	4.6 x 10
Max. operating pressure (psi/MPa/Bar)	200/1.3/14	200/1.3/14

Preparation for Initial Use

The columns are supplied in a storage buffer of 0.1 M NaCl + 20% ethanol. The counter ion for the Q column is Cl^- and Na^+ for the S column. Prior to initial use and after extended storage periods, each column should be conditioned as described below (steps 1–4). Always use HPLC grade reagents and filter and degas the buffers. During this operation do not exceed flow rates of **0.5 ml/min**.

1. Wash with 5 ml of water. Elevate backpressures may occur when washing with deionized water. Do not exceed 300 psi
2. Wash with 5 ml of low ionic strength start buffer [*e.g.* 20 mM Tris-HCl (Q) or 20 mM Sodium Phosphate (S)].
3. Wash with 5 ml of high ionic strength buffer (*e.g.* Starting buffer + 1.0 M NaCl).
4. Wash with 5 ml of low ionic strength start buffer [*e.g.* 20 mM Tris-HCl (Q) or 20 mM Sodium Phosphate (S)].

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

Buffer Selection

Table 2 and 3 lists commonly-used buffers for anion and cation-exchange chromatography. The choice of whether to use an anion- or cation exchanger is determined mainly by (a) the isoelectric point (pI) and, (b) the relationship between pH and the activity/stability of the protein of interest. Once the form of ion-exchanger is determined, the choice of buffer and pH is also determined by the pH-activity relationship. As a general rule, the pH used should be within ± 0.5 units of the pK_a of the chosen buffer. This permits use of the lowest possible buffer concentration

while maintaining maximum buffering capacity. In any case, a buffer concentration of 20 mM is recommended.

As can be seen in Table 2 and 3, the pK_a and hence the pH of the buffer, changes with temperature. Therefore the pH of the buffer must be adjusted at the working temperature.

Table 2. Buffers for Anion-Exchange Chromatography

pH range	Buffer	Mwt	pK_a @25 °C	Counter-ion	ΔpK_a/°C
5.0 - 6.0	Piperazine	86.1	5.7	Cl^- /HCOO-	-0.015
5.5 - 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, 9.0	Cl^-	
7.3 - 8.3	Triethanolamine	149.2	7.8	Cl^- /CH_3COO^-	-0.020
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^-	-0.026

Table 3. Buffers for Cation-Exchange Chromatography

pH range	Buffer	Mwt	pK_a @25 °C	Counter-ion	ΔpK_a/°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 (Monobasic) 142 (Dibasic)	7.2	Na^+	-0.003
6.8 - 8.2	TES	229.2	7.4	Na^+	-0.020
6.8 - 8.2	HEPES	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.

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 accomplished using Bio-Spin® 6 or Bio-Spin 30 columns, Econo-Pac® 10DG desalting columns, Bio-Gel® P-6DG size exclusion gel or the Econo-Pac P6 cartridge. The choice of product depends on sample volume. Always centrifuge or filter the sample (0.2–0.45 mm filter) to remove particulates. Application of turbid or lipid-containing samples may reduce the column lifetime.

Sample Load

The recommended 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, either change to the UNO 1, 6, or 12 ml columns or perform several chromatographic runs with a reduced loading. 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.

Choice of Elution Salt

Sodium or potassium chloride are the most common elution salts and are recommended for use with UNO columns. Other ions may be used and may show different selectivities based on their relative elution strengths and chaotropic nature.

The following ions are shown below in order of elution strengths:

Cations for UNO S Columns

Barium > Calcium > Magnesium > Potassium > Sodium > Lithium

Anions for UNO Q Columns

Citrate > Sulfate > Iodide > Chloride > Formate > Acetate

See Reference 1 for a more detailed explanation of ion selectivity in chromatographic separations.

Gradient Volumes & Salt Concentrations

As a starting point for developing a separation, we recommend using the UNO Q Polishing column with a simple gradient profile over 2–6 ml.

Protocol: Use a flow-rate of 0.5 ml/min. Following sample application, wash unbound proteins from the column with 2 ml of Start Buffer A. For elution, use a gradient volume of 6 ml to a Cl^- concentration of 0.5 M (50% B). Follow this segment of the gradient by raising the salt concentration to 1.0 M (100% B) over 2 ml and then hold at 1.0 M for 2 ml before re-equilibrating the column with 6 ml of start buffer A. This gradient is shown schematically in Figure 1. Once an initial separation has been performed and the elution position of the protein of interest determined, the gradient composition and volume is adjusted to achieve maximum resolution. Normally, a gradient volume of 2 to 6 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.

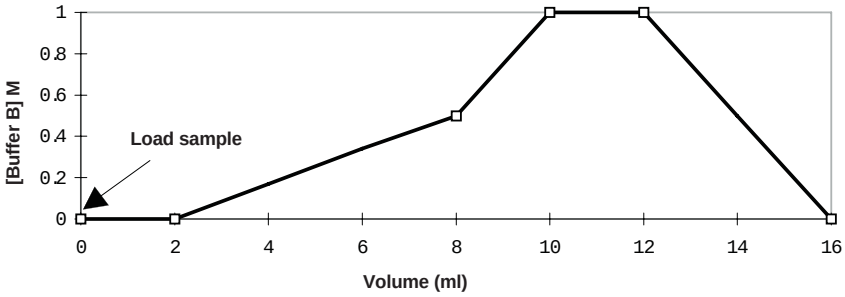

Fig. 1 Schematic gradient for separation on UNO Polishing column.

Use of Detergents

Cationic or non-ionic detergents may be used with the UNO Q support. Anionic or non-ionic detergents may be used with the UNO S support. We recommend the use of the reduced form of Triton X-100 to minimize UV absorption artifacts. It is essential to thoroughly equilibrate the column with the detergent-containing buffer prior to sample application. Pay particular attention to pH, which influences the solubility of the various classes of detergents. Problems may arise when using salt gradient elution if the starting conditions include detergent below its critical micelle

concentration (CMC). As the salt concentration (i.e. the counter-ion concentration) increases, the CMC drops and eventually micelles will form. This may cause a sudden increase in the UV baseline as the micelles themselves scatter light. We recommend using a concentration of detergent above the CMC during gradient elution. If subsequent chromatographic steps (*e.g.* hydrophobic interaction) or other biochemical manipulations will be affected by the presence of the detergent, then the initial choice of detergent may be influenced by the availability of a protocol for its removal.

See Reference 2 for a more detailed explanation of the characteristics and use of detergents in biology and biochemistry and their removal from biological samples.

Chromatography Pre-Runs

Prior to loading the sample, it is recommended running a blank gradient to check that the column is clean. In addition, detergents and other buffer components will themselves elute as sharp, UV-absorbing peaks during the gradient, complicating subsequent analysis of the chromatogram.

Column Hygiene

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 (i.e. increasing backpressures or a significant drop in resolution) then a more extensive cleaning protocol as described below (steps 1–5) should be used. **Always reverse the flow during this procedure so tightly-bound substances at the top of the column are quickly removed. This process should be performed at 0.5 ml/min.**

1. Wash with 2 ml of deionized water. Elevated backpressures may occur when washing with deionized water. Do not exceed 300 psi.
2. Wash with 5 ml of 2.0 M NaCl or KCl.
3. Wash with 1 ml of 2.0 M NaOH followed by 1 ml of 0.1 M NaCl.
4. Wash with 1 ml of 50% acetic acid followed by 1 ml of 0.1 M NaCl and 1 ml of deionized water.
5. If lipid contamination is a problem, wash with 1 ml of MeOH followed by 2ml of 0.1 M NaCl.
6. Wash with 5 ml of 2.0 M NaCl or KCl or the salt containing the desired counter-ion.

No repair of this column is possible. If performance deteriorates or backpressures become excessive, then we recommend purchasing a new column.

Storage Conditions

Prior to long-term storage, the column should be cleaned as previously described and then washed with 5 ml of 20% ethanol. This will prevent microbial growth. Store the column in a safe place at room temperature. NEVER allow the column to freeze.

References

1. W. Kopaciewicz, M. A. Rounds, J. Fausnaugh and F. E. Regnier (1983) Retention Model for High-performance Ion-Exchange Chromatography. *J. Chromatography*, **266**, 3-21.
2. A Guide to the Properties and Uses of Detergents in Biology and Biochemistry. J. Neugebauer (Calbiochem Biochemicals).

Product Information

Catalog Number	Product Description
720-0001	**UNO Q-1 Column**
720-0003	**UNO Q-6 Column**
720-0005	**UNO Q-12 Column**
720-0009	**UNO Q Polishing Column**
720-0011	**UNO Q-1 R Column,** replacement column
720-0013	**UNO Q-6 R Column,** replacement column
720-0015	**UNO Q-12 R Column,** replacement column
720-0021	**UNO S-1 Column**
720-0023	**UNO S-6 Column**
720-0025	**UNO S-12 Column**
720-0029	**UNO S Polishing Column**
720-0031	**UNO S-1 R Column,** replacement column
720-0033	**UNO S-6 R Column,** replacement column
720-0035	**UNO S-12 R Column,** replacement column

Catalog Number	Product Description
751-0091	**Bio-Scale 2 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover. Use this kit for the UNO S-1 or Q-1 Column.
751-0095	**Bio-Scale 10 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover. Use this kit for the UNO S-6 or Q-6 Column.
751-0097	**Bio-Scale 20 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover. Use this kit for the UNO S-12 or Q-12 Column.
751-0099	**Bio-Scale Fittings Kit,** includes 2 Super Flangeless Nuts (1/4 x 28 threads) and 6 ferrules, 2 Flangeless M6 Nuts, 4 ferrules and 2 caps, 2 Fingertight II fittings (10–32 threads). Use this kit for any UNO Column.
125-0561	**Anion Exchange Standards,** 6 pack
125-0562	**Cation Exchange Standards,** 6 pack

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