Bio-Scale S Columns

Instruction Manual

Catalog Number
751-0011, 751-0013,
751-0015, 751-0017

BIO-RAD

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Section 1
Characteristics of the Bio-Scale S2, S5, S10, S20 Cation Exchange Columns

1.1 Introduction

Bio-Scale prepacked ion-exchange columns meet the needs of the bio-chromatographer for rapid and reproducible high resolution separations of biomolecules including proteins, peptides, and polynucleotides. The availability of four column sizes (bed volumes of 2, 5, 10, and 20 ml) provides unrivaled flexibility for the economical and predictable scale up of separation and purification protocols without sacrificing resolution due to overloading.

1.2 The MP10 S Separation Medium

Each column contains the spherical, rigid, and highly porous polymeric Macro-Prep® MP10 support derivatized with the strongly acidic $-SO_3^-$ sulfonic acid group. The 10 µm particle size and narrow particle size distribution produces excellent resolution of biomolecules at high flow rates and with very low back-pressures.

The hydrophilic Macro-Prep S support demonstrates extremely low non-specific binding of biomolecules accompanied by high recovery of biological activity.

Stability of the MP10 S Support

The columns are stable over the pH range 2-12, allowing easy cleaning and regeneration. The MP10 support is compatible with aqueous solutions of guanidine-HCl and urea. Non-ionic and anionic detergents and organic solvents such as methanol, ethanol, and isopropanol may also be used.

Table 1. Column Characteristics

	S2	S5	S10	S20
Column volume (ml)	2	5	10	20
Recommended max. protein loading (mg)	20	50	100	200
Recommended flow rates (ml/min)	0.5 to 3.0	0.5 to 5.0	0.5 to 7.0	0.5 to 10.0
Static protein binding capacity (mg HIgG)	100	250	500	1000
Ionic capacity (µmol/ml)	127 ± 25	127 ± 25	127 ± 25	127 ± 25
Average particle size (µm)	10 ± 3.0	10 ± 3.0	10 ± 3.0	10 ± 3.0
Column dimensions (mm)	7 x 52	10 x 64	12 x 88	15 x 113
Maximum operating pressure (psi/bar)	1,000/69	750/50	600/40	500/34

1.3 Connection to the BioLogic, FPLC and HPLC Systems

Each Bio-Scale column is supplied with 0.02 inch ID (1/16 inch OD) Tefzel tubing, 1/4x28 Flangeless Fittings and end caps for connection to the BioLogic system. For connection to HPLC systems, use 10/32 fingertight fittings and M6 fittings for FPLC systems.

Instructions for assembling the fittings are included with the column.

Note: Bio-Rad Laboratories does not recommend or warrant the use of the Bio-Scale columns with solvent delivery systems containing stainless steel parts and used with corrosive eluants containing 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.

Section 2
Use of the Bio-Scale S Columns

2.1 Preparation for Initial Use

The columns are supplied in a storage buffer of 20% ethanol in water. The counter ion is Na^+. 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 filter and degas buffers. During this operation do not exceed more than **25%** of the recommended maximum flowrates (see Table 1).

1. Wash with 5 column volumes of water.
2. Wash with 5 column volumes of low ionic strength equilibration buffer. (*e.g.* 20 millimolar)
3. Wash with 5 column volumes of high ionic strength limit buffer. (*e.g.* 1.0 M molar)
4. Wash with 5 column volumes of low ionic strength equilibration buffer.

The column may now be further equilibrated in the starting buffer at the desired flow rate. Note that buffer additives such as guanidine hydrochloride may cause some shrinkage of the support. This should not affect subsequent chromatography.

2.2 Buffer Selection

Table 2 lists commonly used buffers for cation exchange chromatography. These are anionic or zwitterionic buffers (it is important not to use cationic buffers which would interact with the cationic exchanger).

The choice of whether to use an anion- or cation exchanger is determined mainly by the isoelectric point (p*I*) and the relationship between pH and the activity/stability of the protein of interest. When the type of ion exchanger is determined, the choice of buffer and pH is also determined by the pH activity relationship. As a general rule, the chosen buffer should be used in a pH range of pKa ± 0.5. This permits use of the lowest possible buffer concentration while maintaining maximum buffering capacity. In any case, we recommend a minimum buffer concentration of 20 mM.

As can be seen in Table 2, the pKa, 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 Cation-Exchange Chromatography

pH range	Buffer	MW	pKa @25°C	Counter-ion	Δ 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 (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.

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 accomplished using the Bio-Spin® 6 or Bio-Spin 30 columns, Econo-Pac® 10DG desalting columns, Bio-Gel® P-6DG size exclusion gel, or Econo-Pac P6 cartridge. The choice of product depends on sample volume. Always 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. See Reference 1 for further information about sample preparation.

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 a larger column 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 charged components. This may result in a shift of certain peaks to an earlier elution position in the gradient.

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. For many separations, varying the pH of the elution buffer in addition to its salt concentration may be advantageous. Generally, it is best to choose initial pH and ionic strength conditions such that the protein of interest elutes early in the gradient. This is especially true for labile proteins or where a higher salt concentration is undesirable.

Choice of Elution Salt

NaCl and KCl are the most common elution salts and are recommended for use with Bio-Scale S columns. Other cations may be used and may show different selectivities based on their relative elution strengths and chaotropic nature.

These cations are shown below in order of elution strengths:

Barium > Calcium > Magnesium > Potassium > Sodium > Lithium.

See Reference 2 for a more detailed explanation of ion selectivity.

Gradient Volumes and Salt Concentrations

As a starting point for developing a separation, we recommend using the Bio-Scale S2 column with a simple gradient profile over 48 ml.

Protocol: Use a flow-rate of 2.0 ml/min. Following sample application, wash unbound proteins from the column with 6 ml (3 bed volumes) of Start buffer A. For elution, use a gradient volume of 20 ml (10 bed volumes) to a Na^+ concentration of 0.5M (50% buffer B). Follow this segment of the gradient by raising the salt concentration to 1.0 M (100% B) over 4 ml and then hold at 1.0 M for 6 ml before re-equilibrating the column with 12 ml of 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 is 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.

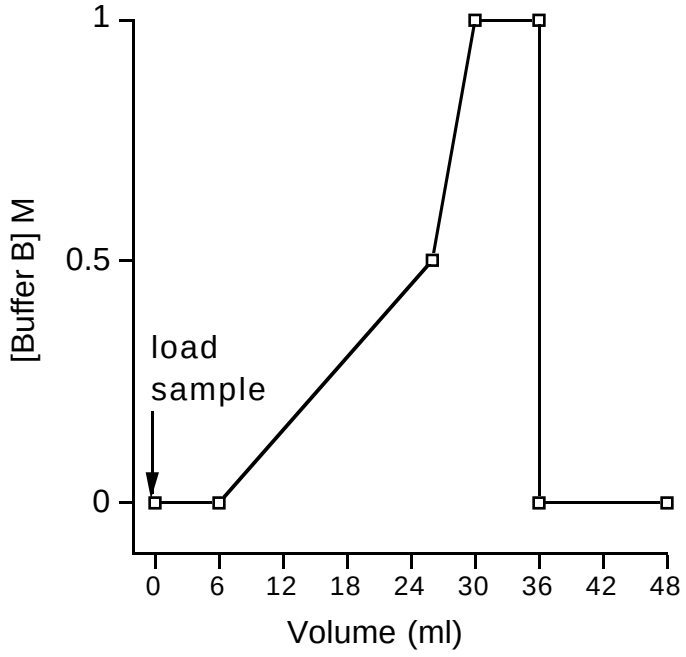

Fig. 1. Schematic gradient for separation on Bio-Scale S2 column.

2.5 Use of Detergents

Anionic or non-ionic detergents may be used with the Bio-Scale 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 anionic 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 (ie the counterion 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 (eg hydrophobic interaction) or other biochemical manipulations will be affected by the presence of the detergent, then the choice of detergent may be influenced by the availability of a protocol for its removal.

See References 1 and 3 for a more detailed explanation of the characteristics and use of detergents in biology and biochemistry and their removal from biological samples.

2.6 Chromatography Pre-Runs

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

2.7 Anomalous Chromatographic Behavior

Certain proteins exhibit anomalous behavior on ion-exchangers due to a non-homogeneous charge distribution on their surface. Inclusion of zwitterions such as taurine and betaine (up to 200 mM) may improve the separation by delocalizing charges on such proteins. See Reference 2 for further information. In addition, proteins of high isoelectric point (pI) may require relatively high salt concentrations for elution. For very tightly-bound proteins, elution may be accomplished using a salt with a higher elution strength. In such cases, elution is often accompanied by a sharpening of the peak profile.

Section 3
Care of the Bio-Scale S Columns

3.1 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 (ie increasing backpressures or a significant drop in resolution) then a more extensive cleaning protocol as described below (steps 1-6) should be used. **Always reverse the flow during this procedure so tightly-bound substances at the top of the column are quickly removed.**

During this operation do not exceed more than **25%** of the recommended maximum 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 2 x 100 µl injections of 1.0 M NaOH followed by 3 column volumes of water.

3) Wash with 2 x 100 µl injections of 50% acetic acid followed by 3 column volumes of water.

4) If lipid contamination is a problem, wash with 1 column volume of methanol or isopropanol 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) Change the top frit, readjust the bed height to eliminate any voids and 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 lock 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 adjust the lock nut to remove the void, retighten the top fitting and resume pumping buffer. If the bed has compressed from long term use, then as a precaution the top frit should be replaced.

Note: The Bio-Scale columns should not be run at their maximum flow-rates for extended periods of time as this will cause excessive bed compression and high backpressures.

3.3 Frit Removal

The top frit may need to be replaced after extensive column use or if increasing backpressures are noticed. Each column is supplied with a Frit Removal Tool, Polyethylene Frit, and Distribution Screen.

The screen assists in distributing the sample over the entire column surface and also acts as a pre-filter for the frit. It should be replaced every time the frit is changed.

Figure 2 describes in detail how to remove and change the top frit.

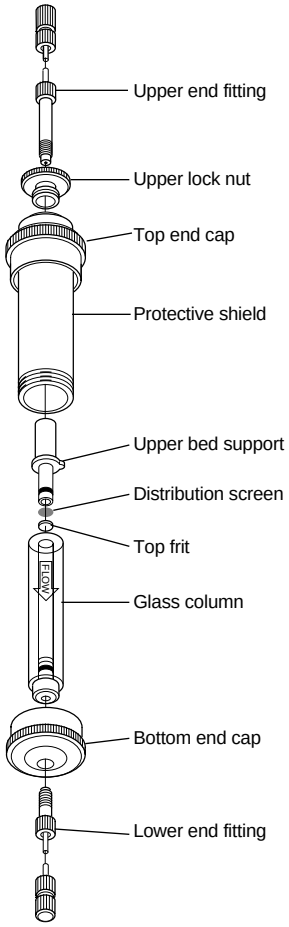

Fig. 2. Instructions for removal of top frit.

1. Remove the upper and lower end fittings from the column.
2. Remove the upper lock nut from the top end cap.
3. Unscrew the bottom end cap from the protective shield and slide out the glass column.
4. Pull out the upper bed support from the glass column.
5. Using the frit removal tool, remove the distribution screen and frit by pressing the hook into the frit in a sideways motion with slight downward pressure.
6. Add 5 ml of buffer to the top of the resin bed. Place a new frit and screen into the tube. Insert the upper bed support and use it to carefully push down the frit and screen until it just touches the top of the resin bed.
7. Insert the glass column through the protective shield, into the top end cap and align the bed support prong with the top end cap guide. Attach the bottom end cap to the shield. Insert the lock nut into the top end cap and twist down until the plunger is at the top of the bed.
8. Attach column to a pump and pump buffer at 2.0 ml/min for 5 to 10 minutes. Inspect the column for voids and adjust accordingly.

3.4 Top-Off Resin

If the top of the resin bed becomes fouled and the above hygiene steps do not restore performance, then a few millimeters of the bed should be removed and replaced with fresh resin obtainable from Bio-Rad.

3.5 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. This will prevent microbial growth. Store the column in a safe place at room temperature. Never allow the column to freeze.

3.6 References

1. Guide to Sample Preparation. Bio-Rad Laboratories Technical Bulletin # 1322.
2. 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.
3. A Guide to the Properties and Uses of Detergents in Biology and Biochemistry J. Neugebauer (Calbiochem Biochemicals).

Section 4
Product Information

Catalog Number	Product Description
751-0011	**Bio-Scale S2 Column**
751-0013	**Bio-Scale S5 Column**
751-0015	**Bio-Scale S10 Column**
751-0017	**Bio-Scale S20 Column**
751-0019	**Top-Off Resin Kit S,** includes 1 ml of MP10 S resin, 5 frits and 5 distribution screens for each column diameter
751-0091	**Bio-Scale 2 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover
751-0093	**Bio-Scale 5 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover
751-0095	**Bio-Scale 10 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover
751-0097	**Bio-Scale 20 Replacement Part Kit,** includes 5 frits, 5 distribution screens, 2 o-rings, 1 frit remover
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)

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