



HRLC[®] MA7S Cation Exchange Columns

Installation and Operation

BIO-RAD

Table of Contents

Section 1	Introduction	1
Section 2	Installation.....	2
2.1	Columns.....	2
Section 3	Operation.....	3
3.1	Equilibration	3
3.2	Preparation of Buffers	4
3.3	Running the Protein Standard.....	4
3.4	Buffers and Ionic Strength Effects	6
3.5	Flow Rate	7
3.6	Sample Application	7
Section 4	Cleaning.....	8
Section 5	Storage	9
Section 6	Product Information.....	11

Section 1

Introduction

HRLC MA7S columns separate biological macromolecules by cation exchange chromatography. In cation exchange chromatography, the fixed negative charges on the packing material interact with the charged biomolecules in the sample. If the biomolecule binds, it can usually be eluted with either step or linear gradients using increasing ionic strength, or by changing the mobile phase, changing the pH, or changing both.

The HRLC MA7S packing is a spherical, pellicular (nonporous), polymeric material. The nonporous nature of the packing material allows high speed, high resolution separations with high recoveries. Since the HRLC MA7S columns or cartridges have no upper exclusion limit due to the pellicular nature of the beads, their use is not limited by the molecular weight of the biomolecules. In addition, chromatography and re-equilibration are extremely fast.

Section 2 Installation

2.1 Columns

When a new column is initially placed on an LC system, the column should be attached only at the inlet end when introducing the mobile phase. This prevents particulates of packing (should the frit have been broken in shipment) or air bubbles (if the column dried during storage) from getting into the detector flow cell. Care should be taken to prevent air from passing through the column by making sure that no bubbles are in the solvent delivery lines in front of the MA7S column. Rinse the 50 x 7.8 mm columns with at least 10 ml HPLC-grade water to remove the shipping solvent. Rinse 100 x 19 mm preparative columns with at least 150 ml of HPLC-grade water.

The column outlet may be attached to the detector when no evidence of a problem is observed. Connect the column outlet to the tubing leading to the detector inlet. To keep band spreading to a minimum, it is important that the tubing between the column and the detector be as short as possible.

Note that all metal tube connections are of the compression screw (reverse nut) type. A ferrule is compressed permanently against the tubing. To insure minimum dead volume, tighten the assembly of tubing, ferrule, and nut fingertight. Push the tubing in until it bottoms firmly. Using a 1/4" wrench, tighten 1/4 turn. The fitting only needs to be tight enough to seal; its lifetime will be diminished by over-tightening.

Section 3 Operation

3.1 Equilibration

To determine whether the HRLC MA7S column or cartridge is performing properly, run the protein standard as described in Section 3.3 before injecting any samples. Before running the standard, or any sample, in a new running buffer, wash the column or cartridge with the high salt buffer for 15 minutes at 1.5 ml/min. Then equilibrate the cartridge for 10 minutes or equilibrate the column for 20 minutes in the low salt buffer at 1.5 ml/min.

3.2 Preparation of Buffers

To make a buffer for the HRLC MA7S columns or cartridges, dissolve the buffer salts in HPLC grade water. Filter the solution through a 0.45 micron or 0.22 micron filter, and degas under vacuum. **Only at this point should pH be adjusted to the desired level.** Because the strengths of the low salt buffers used with the HRLC MA7S packing tend to be very low (typically 20 mM), degassing can significantly affect the buffer pH. We recommend using 2 M NaOH or 6 M HCl to adjust the pH to avoid dilution of the buffer. Buffers should be degassed and the pH checked daily since gases redissolving into the buffers can shift the pH.

3.3 Running the Protein Standard

Buffer Preparation

To make buffer A (20 mM bis-Tris, pH 7.0), dissolve 4.2 g of bis-Tris in 1.0 liter of HPLC grade water. Filter the solution through a 0.45 micron or 0.22 micron filter. Degas thoroughly using a vacuum pump, and adjust the pH to 7.0 with 2 N HCl. Be careful not to overshoot the pH. To make buffer B (20 mM

bis-Tris, 500 mM NaCl, pH 7.0), dissolve 4.2 g of bis-Tris and 29.2 g of NaCl in 1.0 liter of HPLC grade water. Filter, degas, and adjust the pH exactly as described for buffer A.

Standard Preparation

Reconstitute the lyophilized standard by adding 2.0 ml of buffer A. Swirl the vial gently to dissolve all protein. The standard should dissolve completely. If the solution is cloudy or particulates are visible, filter or centrifuge the standard before injecting.

Gradient Program - Column (50 x 7.8 mm)

Flow rate: 1.5 ml/min

Sample volume: 100 μ l

Set up a gradient method for the MA7S column consisting of :

1. 10 minute gradient from 0–50% B;
2. 1 minute hold at 50% B;
3. 0.1 minute step back to 0% B
4. 3 minute re-equilibration into buffer A.

Set the UV monitor to 280 nm, 0.08 AUFS. A chromatogram similar to the enclosed representative chromatogram

should result. If not, wash the column with 0,1 N NaOH (see Cleaning section), re-equilibrate into the running buffers, and inject the standard again.

3.4 Buffers and Ionic Strength Effects

Because the MA7S packing is of low ionic capacity, buffer concentrations must be kept low. Typically, 20 mM buffers are used. Higher strength buffers may prevent the sample from binding to the column. Similarly, if high concentrations of salts are present in the sample solution, dilution, dialysis, or gel filtration may be required to remove excess salts and equilibrate the sample in the starting (low concentration) buffer.

HRLC MA7S columns and cartridges are stable in chaotropes, water-miscible organic solvents, and solvent modifiers typically used in protein HPLC. However, never switch abruptly between aqueous and organic solvent systems. Run a gradient into the chaotrope or solvent at 0.5 ml/min, allowing at least one column volume per 5% change. Try to avoid frequent changes between aqueous and organic solvents. The MA7S packing is stable from pH 2–12.

Always filter and degas the buffers. Filter or centrifuge all samples, too, to remove particulate material.

3.5 Flow Rate

When beginning a chromatographic run, the flow rate should always be increased gradually. An appropriate flow rate should be selected based on resolution and separation time requirements: with the nonporous MA7 matrix, greater resolution is usually achieved with fast flow rates. The table below lists flow rates for the various sizes of cartridges and columns. Operating backpressure should be less than 2,000 psi no matter what the flow rate or column size.

	Column Size
	50 x 7.8 mm
Flow rate (ml/min)	
Recommended range	1.5–10
Maximum	15

3.6 Sample Application

Loading protein in the appropriate range for the size of the column gives the best resolution, but the capacity varies for different proteins. The table below outlines both loading capacity and typical

injection volumes used for cartridges and columns.

	Column Size
	50 x 7.8 mm
Protein loading capacity (mg)	
Static	5–10
Dynamic	1–2
Typical injection volumes (μ l)	100

Section 4 Cleaning

Prolonged operation with complex mixtures may lead to the gradual accumulation of strongly ionic or hydrophobic sample components. These compounds will decrease the resolution and change the retention times of a standard sample. A rise in backpressure is usually indicative of protein or buffer salt precipitation on the column or cartridge. If the backpressure at a given flow rate suddenly rises, lower the flow to keep the pressure below 2,000 psi and rinse with a slow gradient from 0–50% buffer B of the buffer system being used. Hold at 50% B until 5 column volumes have been run through the column. If this doesn't lower the pressure, turn the column or cartridge

around and run it in the opposite flow direction for a few minutes, again using buffer B (the high salt concentration buffer). If this does not decrease the backpressure, the column or cartridge can usually be cleaned using the following procedure.

Wash with 5 column volumes of one or more of the following:

1. Buffered 1.0 M NaCl;
2. 20% (v/v) water miscible organic solvent such as ethanol, methanol, or DMSO.

If these do not lower the backpressure, use the following procedure:

Wash with 0.2 bed volumes of 0.1 N NaOH followed by 5 column volumes of HPLC grade water. Repeat twice if needed.

Re-equilibrate the column or cartridge in buffers A and B for the protein standard and run the protein standard as described in Section 3.3.

Section 5 Storage

After use, MA7S columns should be washed with 0.1 N NaOH as described under "Cleaning" then rinsed with 20 col-

umn volumes of HPLC-grade water. If the column or cartridge is to be run the next day, it can be left on the HPLC system in water.

If the HRLC MA7S column or cartridge is to be stored for a longer period, replace the water with 10% ethanol in HPLC-grade water. Remove the column or cartridge from the HPLC system and keep the column ends tightly capped using the screws originally furnished with the column. For cartridges, remove the cartridge from the Standard Cartridge Holder and return it to the plastic tube in which it was shipped. Put 0.25 ml of 10% ethanol in the tube and seal the tube with Parafilm® laboratory film. We recommend storing the columns at 4 °C when not in use. **Do not freeze.** Proper storage as outlined here will prevent drying and bacterial contamination between uses and help to prevent physical damage from occurring to the packing material.

Section 6 Product Information

Catalog Number	Product Description
<i>HRLC MA7 Analytical Columns</i>	
125-0533	HRLC MA7C Weak Cation Exchange Column, 50 x 7.8 mm
125-0534	HRLC MA7P Weak Anion Exchange Column, 50 x 7.8 mm
125-0535	HRLC MA7S Strong Cation Exchange Column, 50 x 7.8 mm
125-0536	HRLC MA7Q Strong Anion Exchange Column, 50 x 7.8 mm
<i>Standards</i>	
125-0561	Protein Standards for Anion Exchange Chromatography, 6 vials of 0.5 ml each
125-0562	Protein Standard for Cation Exchange Chromatography, 6 vials of 0.5 ml each

*Requires Standard Cartridge Holder

Parafilm® is the registered trademark of American Can Company.