



# **HRLC<sup>®</sup> MA7P Anion Exchange Columns and Cartridges: Installation and Operation**

**BIO-RAD**

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# Section 1

## Introduction

HRLC MA7P columns and cartridges separate biological macromolecules by anion exchange chromatography. In anion exchange chromatography, the fixed positive 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, changing the mobile phase, changing the pH, or changing both.

The HRLC MA7P 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 MA7P columns and 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.

# Section 2 Installation

## 2.1 Cartridges

The HRLC MA7P cartridges fit a specially designed HPLC cartridge holder, the Standard Cartridge Holder, catalog number 125-0131. Other holders may not provide optimal results. The holder should be installed on the HPLC system according to the instructions included with it, either before or after the MA7P cartridge is in place. Take care to seat the ferrules to the proper depth for the holder.

To fit the MA7P cartridge into the holder, unscrew one end of the holder and pull it away from the central tube. Loosen the other end a few turns, and then insert the cartridge into the holder, with the flow arrow on the cartridge properly oriented. Firm pressure will pop the cartridge into the holder securely. Fit the free end of the holder onto the cartridge, and screw it down. The holder ends need only be screwed down finger-tight.

Care should be taken to prevent air from passing through the cartridge, by making sure that no bubbles are in the

solvent delivery lines before connecting the Standard Cartridge Holder with the HRLC MA7P cartridge in place. Rinse the MA7P cartridge with at least 5 ml of HPLC-grade water to remove the shipping solvent.

## 2.2 Columns

When a new column is initially placed on an HPLC 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 MA7P column. Rinse the 50 x 7.8 mm columns with at least 10 ml HPLC-grade water to remove the shipping solvent.

After you have completed these steps and made sure that there are no problems, you may attach the column outlet to the detector. Connect the column outlet to the tubing leading to the detector. To keep band spreading to a minimum, it is important that the tubing between the col-

umn 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 MA7P 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 5 minutes or the column for 10 minutes in the low salt buffer at 1.5 ml/min. When using the HRLC MA7P packing,

buffer preparation is especially important. Often, poor or irreproducible results can be traced to improperly made buffers.

### 3.2 Preparation of Buffers

To make a buffer for the HRLC MA7P 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 MA7P 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 Tris, pH 8.5), dissolve 2.42 g of Tris-(hydroxymethyl)-amino-methane\* (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 8.5 with 2 M HCl. Be careful not to overshoot the pH.

To make buffer B (20 mM Tris, 500 mM NaCl, pH 8.5), dissolve 2.42 g of 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  $\mu$ l

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

1. 5 minute gradient from 0–100% B;
2. 1 minute hold at 100% B;
3. 0.1 minute step back to 0% B
4. 3 minute re-equilibration into buffer A.

### 3.4 Buffers and Ionic Strength Effects

Because the MA7P 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. For example, ascites samples, which contain approximately 0.15 M salt, must be diluted 1:4 to bind properly to the column.

HRLC MA7P 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 MA7P 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.

	<b>Column Size</b>
	<b>50 x 7.8 mm</b>
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.

	<b>Column Size</b>
	<b>50 x 7.8 mm</b>
Protein loading capacity (mg)	
Static	5–15
Dynamic	1–2
Typical injection volumes (μl)	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 0–100% buffer B of the buffer system being used. Hold at 100% B for several minutes. If this doesn't lower the pressure, turn the column or cartridge around and run it in the opposite flow direction for at least 5 bed volumes, 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 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, MA7C columns should be washed with 0.1 N NaOH as described in Section 4 then rinsed with 20 column 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 HPLC MA7C column or cartridge is to be stored for a longer period, replace the water with 10% ethanol in HPLC-grade water. Remove the column 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

<b>Catalog Number</b>	<b>Product Description</b>
<b><i>HRLC MA7 Analytical Columns</i></b>	
125-0533	<b>HRLC MA7C Weak Cation Exchange Column, 50 x 7.8 mm</b>
125-0534	<b>HRLC MA7P Weak Anion Exchange Column, 50 x 7.8 mm</b>
125-0535	<b>HRLC MA7S Strong Cation Exchange Column, 50 x 7.8 mm</b>
125-0536	<b>HRLC MA7Q Strong Anion Exchange Column, 50 x 7.8 mm</b>
155-0330	<b>HRLC MA7Q Preparative Column, 100 x 19 mm</b>
<b><i>Standards</i></b>	
125-0561	<b>Protein Standards for Anion Exchange Chromatography, 6 vials of 0.5 ml each</b>
125-0562	<b>Protein Standard for Cation Exchange Chromatography, 6 vials of 0.5 ml each</b>

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\*Requires Standard Cartridge Holder

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