HPLC MA7Q Anion Exchange Columns

Installation and Operation

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Section 1
Introduction

MA7Q columns 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 MA7Q 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 MA7Q columns 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

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 MA7Q column. Rinse the 50 x 7.8 mm column with at least 10 ml HPLC-grade water to remove the shipping solvent.

After you have completed these steps and made sure there are no problems, attach the column outlet to the detector. 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 finger tight. 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 overtightening.

Section 3
Operation

3.1 Equilibration

To determine whether the MA7Q column 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 with the high salt buffer for 15 minutes at 1.5 ml/min. Then equilibrate the column for 10 minutes in the low salt buffer at 1.5 ml/min (preparative columns should be equilibrated for 30 minutes).

When using column 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 MA7Q columns, 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 MA7Q column packings tend to be very low (typically 20 mM), degassing can significantly affect the buffer pH. We recommend using 2 N 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, catalog number 161-0716) 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 N 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. Gently swirl the vial 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: 5 ml/min

Sample volume: 100 µl

Set up a gradient method for the MA7Q 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 MA7Q 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 salt and equilibrate the sample in the starting (low concentration) buffer. For example, ascites samples, which contain approximately 0.15 N salt, must be diluted 1:4 to bind properly to the column.

MA7Q columns 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 MA7Q 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 size columns. Operating backpressure should be less than 2,000 psi no matter what the flow rate or column size.

	Column Sizes 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 Sizes 50 x 7.8 mm
Protein loading capacity range (mg)	
Static	5-10
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 until 5 column volumes have been run through the column. If this doesn't lower the pressure, turn the column 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 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, MA7Q columns should be washed with 0.1 N NaOH as described under Cleaning, then rinsed with 20 column volumes of HPLC-grade water. If the column is to be run the next day, it can be left on the HPLC system in water.

If the column 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. 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
MA7 Analytical Columns	
125-0533	MA7C Weak Cation Exchange Column, 50 x 7.8 mm
125-0534	MA7P Weak Anion Exchange Column, 50 x 7.8 mm
125-0535	MA7S Strong Cation Exchange Column, 50 x 7.8 mm
125-0536	MA7Q Strong Anion Exchange Column, 50 x 7.8 mm
Standards	
125-0561	Protein Standard 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)