HRLC® MP7 HIC Column Installation and Operating Instructions

Catalog Number 125-0509

BIO-RAD

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LIT144 Rev B

Table of Contents

Section 1
Introduction

HRLC MP7 HIC columns separate macromolecules by hydrophobic interaction chromatography. The macromolecules are separated as a result of the interaction between exposed nonpolar and hydrophobic amino acid residues on the protein and the hydrophobic groups on the HPLC column matrix. Unlike reversed phase HPLC which uses strongly hydrophobic groups, the HRLC MP7 HIC column matrix uses a C1 ligand coupled to a polymeric support. This matrix allows elution to be accomplished with aqueous buffers rather than with the organic solvents typically used in reversed phase. The eluants used in hydrophobic interaction chromatography typically do not denature enzymes and proteins.

Section 2
Installation

While unpacking the column, check it carefully for evidence of shipping damage, rough handling, or leaking solvent. Save the shipping container to store the column. If there is evidence of damage,

immediately notify the carrier and your local Bio-Rad Technical Representative.

When installing a new column for use or testing on an LC system, be careful to prevent air from passing through the column by making sure that no bubbles are in the solvent delivery lines in front of the HRLC MP7 HIC column. The column should be connected 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. Rinse the column with HPLC grade water (at least 10 ml) to remove the shipping solvent.

The column outlet can be connected to the detector when no evidence of a problem is observed. It is important that the tubing between the column and the detector be as short as possible.

Please 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

Before injecting any samples onto the column, run the protein standard as described below. Before running the standard, or any sample in a new running buffer, wash the column with the low salt buffer for 15 minutes at 1.0 ml/min. Then equilibrate the column with the high salt buffer at 1.0 ml/min for 15 minutes. We strongly recommend the use of the Bio-Rad HPLC Grade Ammonium Sulfate (catalog number 125-0333) for hydrophobic interaction chromatography. Most commercially available ammonium sulfate absorbs strongly at 280 nm due to various contaminants causing baseline drift. The Bio-Rad HPLC Grade Ammonium Sulfate is of the highest purity available with no detectable protease, deoxyribonuclease, or ribonuclease activity. The optical density of a saturated solution is less than 0.1 at 260 nm.

Section 4
Running the Protein Standard

Prepare 1 liter of each of the following:

a. Buffer A: 1.7 M $(NH_4)_2SO_4$, 0.1 M sodium phosphate, pH 7.0.

b. Buffer B: 0.1 M sodium phosphate, pH 7.0.

Reconstitute lyophilized HIC Protein Standard with 0.5 ml of Buffer A. Shake the vial gently to dissolve all the protein. The standard should dissolve completely. If the solution is cloudy or if particulates are visible, filter or centrifuge the standard before injecting.

Set up a gradient method consisting of:

1. 10 minute gradient from 0 to 100% B;
2. Hold at 100% B for 4 minutes;
3. Step back to 0% B over 1 min;
4. 5 minute re-equilibration with Buffer A.

The flow rate should be at 1.0 ml/min. Set the UV monitor to 280 nm, 0.08 AUFS. Inject 20 microliters of the sample and start the gradient. A chromatogram similar to the enclosed chromatogram should result.

Section 5
Operating Parameters

A. Flow Rate

The flow rate should always be increased gradually. An appropriate flow rate should be selected based on resolution and separation time requirements: greater resolution is generally achieved with low flow rates. The recommended flow rate for the HRLC MP7 HIC column is 1 ml/min. Operating backpressure should be less than 800 psi to maintain column life.

B. Chemical Stability

The HRLC MP7 HIC column matrix is stable from pH 2–12. The column is compatible with reagents such as 0.1% SDS, 1% DTT, 6 M urea (max. flow rate 0.2 ml/min), 10% DMSO, and 100% MeOH. It can be exposed to 1 N NaOH or 0.5 N HCl for short times for cleaning. The column is not compatible with ethanol or acetonitrile. Do not use these solvents with the HRLC MP7 HIC column.

C. Temperature

The operating temperature range of the HRLC MP7 HIC column is 4 °C to 60 °C.

D. Sample Application

Protein loading capacity is generally 5–10 mg per column injection. This loading usually gives the highest resolution, but the capacity varies for different proteins. For example, total capacity for soybean trypin inhibitor is approximately 55 mg and 30 mg for BSA. Recommend injection volume is 20 to 100 microliters.

E. Column Washing

Prolonged operation with complex mixtures may lead to the gradual accumulation of non-eluting sample components. These compounds may decrease the resolution, increase backpressure, or change the retention times of a standard sample.

A sudden rise in column backpressure is usually indicative of protein or buffer salt precipitation on the top of the column. If the backpressure at a given flow rate suddenly rises, lower the flow rate to keep the pressure below 800 psi (56 kg/cm^2) and rinse with the low salt buffer until backpressure decreases. If this does not lower the pressure, turn the column around and run it in the opposite flow direction until the backpressure decreases. Follow elution of the column with the HPLC detector while washing to know when all bound material has been eluted. If the backpressure remains high, use the following recommended wash procedure.

Removal of contaminating compounds can be accomplished by running the column at a low flow rate (0.2 ml/min) with 6 M urea for 1–2 hours, followed by a distilled water rinse (1 hr, 1.0 ml/min).

Section 6
Storage

For overnight storage, keep the column in distilled deionized water. For much longer storage, keep the column in 0.02% sodium azide.

HRLC MP7 HIC column characteristics

Column size	50 x 7.8 mm
Support matrix	polymer
Particle size	7 micron
Pore size	800 angstrom
Optimal flow rate	1.0 ml/min
Maximum flow rate	3.0 ml/min
Normal operating pressure	200 psi (14 kg/cm^2)
Maximum operating pressure	800 psi (56 kg/cm^2)
Temperature range	4–60 °C

Section 7
Product Information

Catalog Number	Product Description
	HRLC MA7 Cartridges
125-0509	**HRLC MP7 HIC Column,** 50 x 7.8 mm
125-0559	**MP7 HIC Protein Standard Test Mixture**
125-0333	**HPLC Grade Ammonium Sulfate,** 500 g