Hi-Pore® Reversed Phase Column Installation and Operation

Introduction

The Hi-Pore column packing material has been batch tested for protein separations and the packed column has been tested for packing efficiency. If you have any questions about the performance or application of this column, please contact your Bio-Rad representative.

Column Preparation and Installation

For best results with your new Hi-Pore column, connect the column using the enclosed fittings.

To insure maximum shelf life and to protect the column from freezing, each Hi-Pore column is equilibrated with methanol after testing. Prime your HPLC system with methanol or acetonitrile before connecting the column. Connect the column to your HPLC system in the following manner:

1. Remove the plug from the inlet end of the column (refer to the flow direction arrow printed on the column tag).
2. Connect the inlet tubing to the column. Tighten with a wrench, only until snug.
3. Remove the plug from the outlet end of the column.
4. Pump 30 ml of degassed solvent through the column to clear any air bubbles.
5. Connect the outlet tubing to the column.
6. Equilibrate the column with your eluant. In gradient systems, use the starting eluant.

In switching to buffers from any organic solvent, run a gradient from 0% buffer to 100% buffer over a 30 minute period. This prevents precipitation of salts in the organic solvent.

Operating Conditions

1. The column pressure cannot exceed 5,000 psi.
2. The pH range is 2 to 7.
3. Do not use eluants containing fluoride salts.

Tips on Column Use

Solvent Changes

When changing solvents, use miscible solvents. A change from acetonitrile to aqueous buffer should use water as an intermediate step to avoid precipitation of buffer salts.

Temperature Control

Because sample retention changes as a function of temperature, temperature control can be used in optimizing separations. Lack of temperature control can contribute to variability in sample retention.

Solvent Purity

An often-overlooked cause of unsatisfactory HPLC performance is the presence of trace impurities in the solvent. These can contribute to baseline instability (fluorescent or UV absorbing components), altered retention times (especially true of trace water in absorption chromatography), or even irretrievably ruin column performance. Many solvent suppliers offer an LC or distilled in glass grade of solvent which is suitable for use in HPLC.

Storage

Columns should be stored in acetonitrile if not used frequently. If eluants contain salts, wash the column with water before rinsing with acetonitrile for storage.

If column performance degrades in spite of appropriate preventive steps, the following procedures may restore the column to its original performance:

1. If column back-pressure is high, disconnect the column from the injector and run the pumps to verify that the back-pressure is due to the column and not the pumping system or tubing.
2. If damaging material has accumulated at the top of the column, reverse solvent flow through the column to attempt to wash it off. Use low flow rates (0.5 ml/min) in reversed flow.
3. If small, very hydrophobic molecules are being chromatographed, the column may be cleaned by pumping 100-200 ml of a very non-polar solvent such as methylene chloride or chloroform through the column. This will often elute very non-polar contaminants and restore column performance.

4. If biological materials are being chromatographed, loss of column performance may be due to protein not being eluted from the column. This may occur because the protein is very hydrophobic and the solvents used do not elute it, or because proteins often elute only in a narrow window of organic solvent. In the latter case, protein is retained at both lower and higher concentrations of organic solvent. To try to elute both kinds of retained protein, run a gradient from 25 - 100% non-polar solvent, where the polar solvent is 0.1% TFA in water and the non-polar solvent is 0.1% TFA in 1:2 acetonitrile:isopropanol.
5. If biological molecules are being chromatographed and recovery is poor or peaks are unusually broad, the column may be overloaded or the sample may not be sufficiently soluble in the solvent being used. Inject smaller samples or use a different solvent and see if performance improves. Large or very hydrophobic proteins more easily overload columns and give lower recoveries than small or less hydrophobic proteins.
6. If protein appears to be irreversibly retained on the column after the above steps, wash the column with 1:4 mixture of 0.1 N HNO_3:isopropanol, preferably at an elevated temperature (60-80 ˚C). Pump this solvent mixture through the column for several hours (overnight) at a low flow rate. This procedure may hydrolyze protein remaining on the column. The column may then be washed by running a standard solvent gradient.
7. If the above procedures fail to restore column performance, contact Bio-Rad Laboratories. Prior to contacting Bio-Rad Laboratories, repeat the analysis used in the QC selectivity test using one or more of the standard compounds. Results of this test will help us diagnose the problem and assist you in restoring column performance.