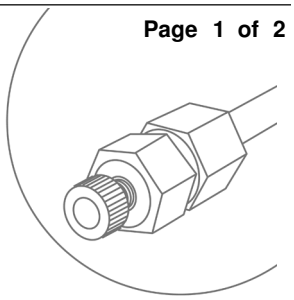
HPLC Column Care Note

Yarra 1.8 µm



Mfr. Name:	Phenomenex
Technique:	HPLC
Care ID:	57
Care Group:	Yarra 1.8 µm
Information Source:	Phenomenex
pH Range:	1.5-8.5
Guard Column:	N/A
Update:	10/8/2015

Applies To

Phenomenex 1.8 µm Gel Filtration /Size Exclusion silica column (Yarra SEC-X150)

Introduction

Each column manufactured by Phenomenex is individually prepared and tested. Every column is supplied with a Test Chromatogram and Specification Sheet, which indicates testing conditions, operating parameters, and column identity details. The Column Details, including specifications and performance test results should be entered into an appropriate information management system for easy tracking and reference should a problem arise.

Inspection

Upon Receipt of the Column:
Verify the column you received is the one you ordered
Check the column for physical damage caused by shipping
Test the column IMMEDIATELY to verify performance.
Record the result of your test in your Column Information Management system. Column Test Standard Aqueous SEC 1 P/N AL0-3042

Installation

Flush your HPLC pump and line with the filtered and degassed mobile phase advised in column test procedure. Ensure no air bubbles are trapped in the system. Set the flow rate to 0.1mL/min and connect to the column inlet, ensuring that flow is in the direction indicated by the arrow on the column. Tighten the end fitting going into the column and slowly increase flow to 0.35 mL/min or method flow-rate for 5 minutes. Stop flow and wipe outlet end of column to remove any particulates prior to connecting to the flow cell. Connect column outlet to flow cell and pass approximately 5 column volumes through the system at 0.35 mL/min while observing the back pressure. A steady back pressure indicates constant flow - while fluctuations may indicate air in the system. Wide fluctuations may shock and damage the column. When a steady pressure has been attained within the prescribed limits, the column is ready for use.

New columns should be tested with GFC test mix, AL0-3042 and previously used columns should be tested on a regular basis. Remember to re-equilibrate the system when changing solvents. Never change to (or from) a buffer/salt solution where the buffer/salt is not soluble in the second solvent. Again this will damage the column. Never attempt to remove the column end fittings. This will void the warranty.

Flow Rate

Adjust to maintain within pressure limits. Typical flow rates for 4.6 mm SEC columns are between 0.3-0.35 mL/min. Do not exceed 0.4 mL/min for Yarra SEC-X150 GFC columns.

Pressure

Typical operating backpressures are between 2800-3600 PSI. Do not exceed 7000 PSI for Yarra SEC-X150.

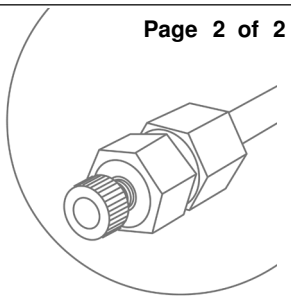
Temperature

50°C Max for Yarra SEC-X150 Columns.

Avoid

pH <1.5 will strip the bonded functional groups. pH >8.5 will dissolve the silica.
Immiscible solvents and buffers; flush with 100% water to remove any buffer salts prior to solvent changes
Avoid trace impurities by centrifuging or filtering sample.

Sudden pressure changes can void column.



Mobile Phase

Use only highest purity chemicals and reagents.
Trace impurities can dramatically degrade column life.
Degas and filter all mobile phases prior to use.
Check for miscibility/solubility when changing solvents and buffers.
Ensure sample (and matrix) are completely soluble/miscible with mobile phase.
Immiscible solvents or salt precipitation can permanently damage the column.

Sample Prep

Check for sample solubility in mobile phase. Use mobile phase as diluent where possible and try to best match diluent viscosity with mobile phase to minimize "solvent fingering" that can reduce performance. Trace impurities can dramatically degrade column life. Filter all samples using a 0.2µ porosity filter prior to injection. Additionally, an inline filter will prevent particulates from fouling the column.

Column Conditioning

Be sure the system is first thermally and electronically stable, especially the electronic (background) noise from the detector. Condition the column at the operating flow rate until a stable baseline is observed.

Column Cleaning

To clean a 150mm x 4.6mm column:
General rinse: 2.5-5.0 mL of 0.1 M Na2HPO4 buffer, at pH 3.0, Follow with a rinse of 1-2 mL with water

Hydrophobic protein removal:
Run gradient 0.1 mL/min using A:Water, B:ACN 0% to 100% B over 30 min followed by100% to 0% B over 30 min
Make sure to flush with 100% water before starting ACN gradient to avoid precipitation of any buffer salts.

Removal of strongly adsorbed proteins:
Wash with 2.5-5.0 mL of 0.5% SDS or 6M Guanidine thiocyanate, or 10% DMSO
Immediately wash with water overnight.

Regeneration

After exposure to denaturants (e.g. SDS, Guanidine thiosulfate, urea), wash with water overnight. Test column with Aqueous SEC 1 Standard (AL0-3042) after denaturants to verify column performance.

Storage

Column storage conditions affect column life time.
Overnight Storage, run mobile phase at <0.1 mL/min.
Prolonged Storage: Column can be stored with 0.1 M NaH2PO4/ 0.025% NaN3 in water or 20% Methanol in water. Flush column with approximately 5 column volumes of 100% water when switching between mobile phase and storage solvent.

Mechanical Shock

Handle columns with care. Do not drop or create physical shock. Do not start pump at high flow rates instead ramp up gradually over a few minutes. Set your pump pressure limit to protect the column in event of blockage. This can create voids which will detrimentally affect the column's performance.

Testing Column

Test Mix: AQUEOUS SEC 1
Order No.: AL0-3042
Bovine gamma globulin, Bovine thyroglobulin, Ovalbumin, Myoglobin, Uridine (Reconstitute in 1mL of phosphate Buffer pH 6.8)
17g Na2HPO4 + 13g NaH2PO4 in Water,0.35 mL/min, UV@280nm
1 µL injected for 4.6 mm ID column.

Warranty

Phenomenex HPLC columns are warranted to meet the stated performance and quality and to be free of defects in material and workmanship. If you are unsatisfied for any reason, please give your Phenomenex Technical Representative a call. We'll do our best to solve the problem to your satisfaction. Should it become necessary to return the column, a Return Authorization Number must be obtained from Phenomenex first.