



Yarra® HPLC/UHPLC Columns

Tips for Care and Use

General Information

Each Yarra column manufactured by Phenomenex is individually prepared and tested. Every column is supplied with a Certificate of Quality Assurance (CQA) which indicates testing conditions, operating parameters, and column details. The column details, including specifications and performance test results should be entered into your information management system for easy tracking and reference. Electronic copies of your column's quality documentation can also be acquired at: www.phenomenex.com/mysupport.

Inspection

Upon receipt of column, please verify that the column you received is the one you ordered (i.e. dimension, particle size, media). Additionally, please check the column for any physical damage potentially caused during shipment. Test the column immediately to verify performance and record the result of your test in your column information management system.

Column Characteristics

	SEC-X150	SEC-X300	SEC-2000	SEC-3000	SEC-4000	SEC-2000 PREP	SEC-3000 PREP	SEC-4000 PREP
Resin Type	Silica	Silica	Silica	Silica	Silica	Silica	Silica	Silica
Particle Size (µm)	1.8	1.8	3	3	3	5	5	5
Pore Size (Å)	150	300	145	290	500	145	290	500
MW Range (Da)	1K-450K	10K-700K	1K-300K	5K-700K	15K-1500K	1K-300K	5K-700K	15K-1500K
pH Range	1.5 - 8.5	1.5 - 8.5	2.5 - 7.5	2.5 - 7.5	2.5 - 7.5	2.5 - 7.5	2.5 - 7.5	2.5 - 7.5
Typical Backpressure (psi)	3,250	3,250	1,300	1,300	1,000	800	800	800
Max Backpressure (psi)	7,000	7,000	3,000	3,000	1,700	1,500	1,500	1,500
Typical Efficiency	> 30,000 (150 x 4.6 mm)	> 30,000 (150 x 4.6 mm)	48,000 (300 x 7.8 mm)	48,000 (300 x 7.8 mm)	38,000 (300 x 7.8 mm)	30,000 (300 x 21.2 mm)	30,000 (300 x 21.2 mm)	30,000 (300 x 21.2 mm)
Max. Flow Rate (mL/min)	0.4*	0.4*	1.5	1.5	1.2	10	10	10

* 0.4 mL/min for 150 x 4.6 mm
0.35 mL/min for 300 x 4.6 mm

Shipping Solvent

Unless otherwise noted on column tag, Yarra SEC columns are shipped in 0.1 M Phosphate Buffer pH 6.8 with 0.025% NaN₃.

Max Temperature:

- Suggested max temperature for Yarra SEC columns is 50°C, however temperature limits are dependent on your running parameters. Running at a pH greater than 8 at 50°C will compromise column lifetime.
- Continuous use of Yarra columns at the maximum temperature limit may compromise column longevity.



Phenomenex products are available worldwide.

www.phenomenex.com/mysupport

QUALITY
MANAGEMENT SYSTEM
CERTIFIED BY DNV GL
= 9001:2008 =

Mobile Phase Compatibility

Use

1. Highest purity grade chemicals and reagents.
2. Filtered and degassed mobile phase.
3. Sample, buffer and reagent that are miscible with the buffer and the reagents on the system.
4. Use mobile phase to dilute sample when possible.

Avoid

1. Immiscible salts as salt precipitation can permanently damage the column.
2. Working outside of the pH range of each Yarra SEC column will cause harm to the silica and phase.
3. Avoid using greater than 20% organic solvent. Even though the column can tolerate high concentration of organic, salt or protein precipitation may occur.

Column Installation

- Initial set-up of your LC system is very important to ensure column performance.

Check that your LC system is ready:

1. Seals, lines, injector clean
2. Lines primed (no dry lines or bubbles)
3. Steady baseline
4. Consistent pressures

- Flush LC system pump and line with mobile phase (HPLC grade and miscible with solvents that column is shipped in).

Mobile phase starting conditions check list:

1. Ensure that HPLC grade mobile phase is well mixed, filtered, and degassed prior to use.
2. Ensure that column shipping solvent, remaining solvent in LC system, and mobile phase solvents are miscible.

- Set flow rate to 0.1 mL/min (for 2.1-4.6 mm ID) and install the column making sure that the arrow is in the direction of flow. Then increase the flow rate to 0.2 mL/min (2.1mm ID) or 1.0 mL/min (4.6 mm ID) for 5-10 minutes. Collect solvent in a small beaker.
- Stop flow and wipe outlet end of column to remove any particulates before connecting to detector.
- Install fitting/tubing into outlet end and run minimum 10 column volumes at low flow (~0.2 mL/min) while monitoring the backpressure.
 1. A steady pressure should indicate a constant flow while pressure fluctuation will indicate air in the system.
 2. Wide fluctuations in pressure may shock and damage the column so it's important to monitor the pressure.
- Monitor pressure as well as signal from the detector, when both are steady, the column is ready for use.

Testing Column Performance

When testing column performance, please use the manufacturer approved test mix.

Aqueous SEC	
Name:	Aqueous SEC 1 Test Mix
Part No.:	AL0-3042
Contents:	Bovine thyroglobulin; Human gamma globulin (contains IgA and IgG); Ovalbumin; Myoglobin; Uridine (reconstitute with 1 mL of 100 mM Sodium Phosphate pH 6.8)
Solvent:	100 mM Sodium phosphate, pH 6.8
Detection:	UV @ 280 nm
Injection Vol.:	Depends on dimension

Column Cleaning

General Cleaning Procedure:

- Start with 10 column volumes of 0.1 M NaH₂PO₄ buffer, pH 3.0.
- Follow with at least 10 column volumes of 100% HPLC grade water.
- Reverse flushing is acceptable, but please reduce flow rate to low flow (eg. 1 mL/min method flow to 0.5 mL/min).

Hydrophobic Analytes:

- Set gradient clean from 100% water to 100% acetonitrile for 30 mins, at low flow.
- Then gradient clean from 100% acetonitrile to 100% water for 30 mins, at low flow.

Removal of Strongly Adsorbed Proteins:

- Wash with 30 mL of 0.5% SDS, 6M Guanidine thiocyanate or 10% DMSO for 30 mins at a reduced flow rate.
- Immediately flush with water overnight at low flow.

Column Regeneration

After exposure to denaturants (SDS, Guanidine thiosulfate, urea), flush with water overnight at low flow. Test column with Aqueous SEC 1 Standard (AL0-3042) after denaturants to verify column performance. Then trial inject your standard to further confirm.

Column Storage

It is very important to make sure that your column is clean before storage. This includes removal of buffer, salts, sample, and ion-pairing agents.

The recommended storage conditions are:

- Overnight storage: run mobile phase at 0.2 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.

Tips for Extending Column Lifetime

- Filter samples with 0.2 or 0.45 μm syringe filters (PhenexTM) to minimize the injection of unwanted contaminants onto your system and column.
- Use the correct guard column or guard cartridge system (SecurityGuardTM) to help remove particulates before they foul your column.
- Do not overload your column. Inject suitable sample concentrations and volumes.
- Work in the appropriate separation mode for the column. Please see column characteristic chart for typical modes each stationary phase is used for.
- Store your column in appropriate solvent(s).

Column Warranties

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.

Disclaimers

New columns should be tested with the manufacturers recommended test mix, and previously used columns should be tested with the same or a suitable test mix for the analysis. Remember to re-equilibrate the system when changing solvents. Never change from one solvent to another which is immiscible, without going through an intermediate solvent which is miscible with both. This will damage the column. 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.

Column 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.

Column Questions and Support

If you have any additional questions, please reach out to our amazing technical team through:

Email: support@phxtechnical.zendesk.com

Live Chat: <https://www.phenomenex.com/chat>

For more information on Yarra SEC columns, please visit www.phenomenex.com/Yarra

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