Lux HPLC Columns
Tips for Care and Use

General Information
Each Lux 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

<table>
<thead>
<tr>
<th>Lux Phase</th>
<th>Chiral Selector</th>
<th>Particle Sizes (µm)</th>
<th>Pore Size (Å)</th>
<th>pH Stability</th>
<th>Shipping Solvent</th>
<th>Suggested Modes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose-1</td>
<td>Cellulose tris(3,5-dimethylphenylcarbamate)</td>
<td>3, 5, 10, 20</td>
<td>1000</td>
<td>2-9</td>
<td>n-Hexane/2-propanol (90:10)</td>
<td>Normal Phase Polar Organic Reversed Phase SFC</td>
</tr>
<tr>
<td>Cellulose-2</td>
<td>Cellulose tris(3-chloro-4-methylphenylcarbamate)</td>
<td>3, 5, 10, 20</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose-3</td>
<td>Cellulose tris(4-methylbenzoate)</td>
<td>3, 5, 10, 20</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose-4</td>
<td>Cellulose tris(4-chloro-3-methylphenylcarbamate)</td>
<td>3, 5, 10, 20</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylose-1</td>
<td>Amylose tris(3,5-dimethylphenylcarbamate)</td>
<td>3, 5</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylose-2</td>
<td>Amylose tris(5-chloro-2-methylphenylcarbamate)</td>
<td>3, 5</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i-Cellulose-5</td>
<td>Cellulose tris(3,5-dichlorophenylcarbamate)</td>
<td>3, 5</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i-Amylose-1</td>
<td>Amylose tris(3,5-dimethylphenylcarbamate)</td>
<td>3, 5</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i-Amylose-3</td>
<td>Amylose tris(3-chloro-5-methylphenylcarbamate)</td>
<td>3, 5</td>
<td>1000</td>
<td>2-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>Proprietary</td>
<td>3</td>
<td>1000</td>
<td>1-11.5</td>
<td>Acetonitrile/Water (85:15)</td>
<td>Reversed Phase</td>
</tr>
</tbody>
</table>

Column Installation
Initial setup of your LC system is very important to ensure column performance:

Ensure 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 (2.1 mm 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.

Phenomenex products are available worldwide.
www.phenomenex.com/mysupport
SFC Column Installation
1. Install the column in the SFC instrument oven compartment
2. Set SFC instrument backpressure regulator between 80-100 bar and equilibrate the column with a minimum of ten column volumes of the SFC mobile phase prior to use.
3. A good starting choice for SFC mobile phase is CO₂/Methanol or CO₂/Ethanol (80:20, v/v) with or without additives.
4. Optimal flow rate for 4.6mm ID columns is between 3 and 6mL/min. We recommend increasing flow rate gradually to 3mL/min to prevent backpressure to go above 300 bar (4300 psi).

Solvent Switching
An appropriate column washing procedure must be applied when changing from one mobile phase to another. The miscibility of the different mobile phase components must be carefully considered for this wash. Lux coated columns are recommended to be dedicated to either normal phase, reverse phase or polar organic. It is not recommended to switch back and forth between different operating conditions.

Normal Phase to Polar Organic or Reversed Phase
To safely transfer a column from normal phase to polar organic or reversed phase conditions, use the following procedure:
1. Set the flow rate to 0.5mL/min
2. Flush the column with 10 column volumes of methanol/ethanol (90:10 v/v)
   a. Eg. 25mL for 250 x 4.6mm column
3. Condition the column with at least 10 column volumes of the new mobile phase.

**If the salts of your reversed phase mobile phase buffer are insoluble in methanol and/or ethanol, flush column briefly with water following methanol/ethanol step before conditioning with 10 column volumes of reversed phase or polar organic mobile phase.

Reversed Phase to Normal Phase
Once a Lux column (except for i-Amylose-1, i-Amylose-3, and i-Cellulose-5) is in reversed phase mode, it is not recommended to switch from reversed phase back to normal phase mode.

Polar Organic to Normal Phase
Once a Lux column (except for i-Amylose-1, i-Amylose-3, and i-Cellulose-5) is in polar organic mode, it is not recommended to switch to normal phase mode.

Normal Phase or Polar Organic or Reversed Phase to SFC
1. Use methanol/ethanol (90:10 v/v) and set the flow to 0.5mL/min.
2. Flush for at least 10 column volumes before switching to CO₂.
3. When conditioning using SFC solvents, lower the flow rate to 0.3mL/min until the methanol/ethanol flushed out.

SFC to Normal Phase or Polar Organic or Reversed Phase:
1. Use methanol/ethanol (90:10 v/v) and set the flow to 0.5mL/min.
2. Flush for at least 10 column volumes until all the CO₂ has been purged.
3. Then condition for at least 10 column volumes with the new mobile phase.

Typical Flow Rate, Backpressure, Temperature:
Here are some typical values for common dimensions of Lux HPLC columns. These numbers are not absolute values as they can differ based on LC system, running parameters and sample analytes/matrix. The values below have been created using a solvent system of Hexane and IPA.

<table>
<thead>
<tr>
<th>Particle Size (µm)</th>
<th>Internal Diameter (ID)</th>
<th>Typical Flow</th>
<th>Typical Pressure (PSI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>0.2</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.4</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
<td>0.5</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>4.6</td>
<td>0.5</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>2.5</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>21.2</td>
<td>20</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>40</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>50</td>
<td>750</td>
</tr>
</tbody>
</table>

Max Backpressure
- Suggested max pressure for Lux LC analytical columns is 4500 psi (310 bar) and AXIA™ prep columns is 3625 psi (250 bar), however pressure limits are dependent on your running parameters and system may vary.

Max Temperature:
- Suggested max temperature for Lux LC columns is 50°C, however temperature limits are dependent on your running parameters and may vary.

Mobile Phase Compatibility
Lux chiral stationary phases (except i-Cellulose-5, i-Amylose-3, and i-Amylose-1) are prepared by coating the silica with a polysaccharide derivative. Therefore, any solvent that can dissolve the polysaccharide derivative, such as mentioned below, must be avoided even in trace amounts:
- Tetrahydrofuran, acetone, chlorinated hydrocarbons, ethyl acetate, dimethysulfoxide, dimethylformamide, N-methylformamide, tolune, methyl ethyl ketone, methyl tert-butyl ether, etc.
- The immobilized portion of the Lux i-Cellulose-5, i-Amylose-3, and i-Amylose-1 greatly increases column robustness by tolerating strong organic solvents such as DMSO, DCM, Ethyl Acetate, MIBE, and THF to be injected onto the column.

Lux columns will deliver consistent results when operated with mobile phases containing additives at the concentration levels specified below. However, limited decrease in column efficiency may occur when a column is used in combination with these additives. Therefore, we advise to dedicate columns to mobile phases containing basic additives.

For basic samples or acidic chiral compounds, it may be necessary to use an appropriate mobile phase modifier in order to achieve chiral resolution and to ensure proper peak shapes.

1. Diethylamine, ethanolamine and butyl amine (0.1-0.5 %) can be used with basic analytes.
2. Trifluoroacetic or acetic acid (0.1-0.2%) with acidic analytes.
3. Mixtures of basic and acidic mobile phase additives are acceptable (i.e. diethyamine acetate or trifluoroacetate).

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:
- Reversed phase: Acetonitrile/H₂O (85:15 v/v), Methanol can be used in place of acetonitrile.
- Normal phase: n-Hexane/2-Propanol (9:1 v/v)
Tips for Extending Column Lifetime

- To regenerate or remove potential contaminants after extended use of your Lux column, we recommend flushing the column with 100% methanol or with ethanol for 2-3 hours at the appropriate flow rate. Back flush can also be used to clean the column.
- Utilize sample preparation techniques such as solid phase extraction (Strata®-X SPE products) or accessories (Phenex™ Syringe Filters) to minimize the injection of unwanted contaminants onto your system and column.
- Use the correct guard column or guard cartridge system (Security-Guard™) to help remove particulates before they foul your column.
- Do not overload your column. Inject suitable sample concentrations and volumes. See chart: Typical Loading Capacities
- 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).
- Solvent switch correctly by slowly acclimating the phase from one miscible solvent to the other at a low flow: 0.1 mL/min for 2.1 mm ID and 0.5 mL/min for 4.6 mm ID.

Testing Column Performance

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

**Normal Phase**
- Name: Chiral Test Mix 5
- Part No.: AL0-8412
- Unit Capacity: 2 mL
- Contents: trans-Stilbene oxide, 0.5 mg/mL, CAS [1439-07-2]
- Diluent: Hexane/Isopropanol (90:10)

**Test Conditions**
- Mobile Phase: Hexane/Isopropanol (90:10)
- Flow Rate: 0.5 mL/min
- Injection Vol.: 2.0 μL
- Detection: UV @ 220 nm

Typical Loading Capacities

<table>
<thead>
<tr>
<th>Column Type</th>
<th>ID (mm)</th>
<th>Approx. Dead Volume (mL)</th>
<th>Typical Flow Rate (mL)</th>
<th>Typical and (Max.) Injection Masses (mg)</th>
<th>Typical and (Max.) Injection Volumes (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary (Fused Silica)</td>
<td>0.32</td>
<td>0.0075</td>
<td>0.001 - 0.02</td>
<td>0.001 (0.01)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Microbore</td>
<td>1.0</td>
<td>0.07</td>
<td>0.02 - 0.1</td>
<td>0.01 (0.1)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Analytical</td>
<td>4.6</td>
<td>1.5</td>
<td>0.5 - 2.0</td>
<td>0.1 (2.5)</td>
<td>10 (200)</td>
</tr>
<tr>
<td>Semi-Prep</td>
<td>10.0</td>
<td>7.3</td>
<td>5.0 - 20</td>
<td>1.0 (25)</td>
<td>50 (1000)</td>
</tr>
<tr>
<td>Preparative</td>
<td>20.0</td>
<td>29.2</td>
<td>10 - 200</td>
<td>5.0 (500)</td>
<td>200 (5000)</td>
</tr>
</tbody>
</table>

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:** www.phenomenex.com/chat

For more information on Lux UHPLC, HPLC, and Preparative columns, please visit www.phenomenex.com/Lux

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