User Guide
Aurora Series UHPLC Column

Recommended guidelines for optimal setup and operation of Aurora Series columns:
Part No. AUR2-25075C18A and AUR2-25075C18A-CSI
Part No. AUR2-15075C18A and AUR2-15075C18A-CSI
Part No. AUR2-50150C18A and AUR2-50150C18A-CSI
Part No. 5CMNFSH1 - 5cm AUR Nanospray Flex housing

For more information, visit www.ionopticks.com
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Example Data

**DISCLAIMER:** The use of “we” “us” or “our” in this User Guide are references to IonOpticks Pty Ltd ACN 621 674 459. The information in this User Guide including (without limitation) the recommendations, safety guidelines and product warnings with respect to the use of our products are to be read strictly subject to our terms and conditions which can be located at www.ionopticks.com and the limitations and exclusions of our liability found within those terms. We accept no liability for any loss or damage howsoever or wherever arising (including death and/or personal injury) which results from or is connected with the failure by the customer to use our products strictly in accordance with the directions in this User Guide.
Innovative Design. Transforming Proteomics.

Our columns are differentiated by two key technological advances: a unique packed emitter design that enables maximum mobile phase velocity with no post-column dead volume; and our own nanoZero® technology that provides user friendly ‘plug and play’ connections with true zero pre-column dead volume. Together, these features combine to maximise chromatographic efficiency and dramatically enhance performance, providing a best in class solution for peptide and metabolite LC-MS separations.

Product Features

- Integrated emitter with zero post-column dead volume.
- Pre-fitted with nanoZero® to provide a zero-dead-volume female union between a nanoViper™ Fingertight fitting (or equivalent) and the Aurora series column.
- nanoZero® fitting incorporates a 10-32 internal thread for easy installation.
- Designed to withstand ultra-high-performance LC (UHPLC) backpressures of >1200 bar.
- nanoZero® is electrically conductive to allow a voltage connection to the entrance of the column.

Performance guarantee

Our columns are subjected to rigorous quality control procedures under the direct eye of our Senior Scientists. All IonOpticks products are covered by our 100% performance guarantee. Any item not meeting our high-performance expectations due to manufacturing defects will be replaced without charge to the customer.

Please contact us at support@ionopticks.com if you have any concerns relating to your column. Our terms and conditions are provided with every quote, but we want to make sure you are receiving a quality product every time so please do not hesitate to get in touch with any feedback or concerns.

Compatibility.

Aurora columns are compatible with a wide range of LC-MS systems. Compatibility is not limited to equipment presented in this guide. Please contact support@ionopticks.com for enquiries regarding instrument compatibility.

### UHPLC

<table>
<thead>
<tr>
<th>BRUKER</th>
<th>THERMO SCIENTIFIC</th>
<th>WATERS</th>
</tr>
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<tr>
<td>nanoElute</td>
<td>Dionex UHPLC systems</td>
<td>nanoAcquity</td>
</tr>
<tr>
<td>Easy-nLC 1000/1200</td>
<td></td>
<td>M-Class</td>
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### Mass Spectrometers

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<th>BRUKER</th>
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<tr>
<td>timsTOF Pro</td>
<td>Fusion Eclipse (+/− FAIMS)</td>
</tr>
<tr>
<td>timsTOF fleX</td>
<td>Exploris 480 / 240 / 120 (+/− FAIMS)</td>
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<tr>
<td>maxis II ETD</td>
<td>Orbitrap Fusion</td>
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<tr>
<td>Impact II</td>
<td>Orbitrap Fusion Lumos</td>
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<tr>
<td>Compact</td>
<td>Orbitrap Elite</td>
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<tr>
<td>COLUM Ovens</td>
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<td></td>
<td>LTQ Orbitrap XL</td>
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<tr>
<td>Sonation</td>
<td>Q-Exactive</td>
</tr>
<tr>
<td>Bruker Column Toaster</td>
<td>Q-Exactive HF</td>
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<tr>
<td></td>
<td>Q-Exactive Plus</td>
</tr>
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</table>

Product Specifications.

<table>
<thead>
<tr>
<th>Column format</th>
<th>Analytical column</th>
<th>Pore size</th>
<th>120Å</th>
</tr>
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<tbody>
<tr>
<td>Column type</td>
<td>Reversed-phase</td>
<td>Max pressure</td>
<td>1200 bar</td>
</tr>
<tr>
<td>For use with</td>
<td>UHPLC</td>
<td>Temp limits</td>
<td>60°C (low pH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH stability</td>
<td>1-8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Particle size</td>
<td>1.6µm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stationary phase</td>
<td>C18</td>
</tr>
</tbody>
</table>
Installation of Aurora Series columns.

Setup of UHPLC systems
We recommend operation of Aurora series columns in a one column mode (direct injection). Please refer to manufacturers guidelines for operating your UHPLC in a one column mode.

Handling of fused-silica or glass tubing and tips can result in serious personal injury, including eye and skin injury. Use safety goggles meeting AS/NZS 1336 requirements or equivalent. Puncture and chemical-resistant gloves should also be worn at all times.

Fitting the column to your UHPLC system.
For Bruker CaptiveSpray source.

For optimal usage, please adhere to the following instructions:

**CAUTION** Handling of fused-silica or glass tubing and tips can result in serious personal injury, including eye and skin injury. Use safety goggles meeting AS/NZS 1336 requirements or equivalent. Puncture and chemical-resistant gloves should also be worn at all times.

1. **Unscrew CSI probe from CSI housing**
2. **Disassemble rear metal housing of CSI probe insert (2 screws)**
3. Carefully remove Aurora CSI protective cap.
4. Insert Aurora CSI fitting into CSI probe. Insert using a twisting motion to prevent pinching of rubber seal.
5. Remove the protective plug from the nanoZero® union.
6. Feed Aurora column through the rear metal housing and reassemble CSI probe (2 screws), ensuring rubber seal is in place between metal housing and Aurora CSI fitting.
6.1 Feed Aurora column with nanoZero® fitting through CSI probe housing.
7. While feeding column, use a Kimwipe (with ethanol or IPA), wet both o-ring rubber seals, then screw CSI probe insert into CSI housing before rubber seals dry.
8. The CSI housing is now ready to connect to your LC-MS system.
For Bruker CaptiveSpray source

Retract the column heater from the CaptiveSpray source.

Insert the CSI Housing into the source, press with a small amount of pressure and tighten the screw until finger tight.

Using a gloved finger, block the air inlet and monitor the Fore pressure. The Fore vacuum needs to drop to below 3e-01 mbar within 10 seconds to be considered usable.

If not sealed, try refitting before replacing rubber seals (Refer Bruker CSI troubleshooting guide).

Move the column heater in front of the CaptiveSpray source.

Holding the nanoZero® with a spanner, tighten the nanoViper fitting finger tight until you reach a firm stop. Operation of the column with an under tight fitting can result in a fall in performance that can not be recovered with additional tightening.

Curl the column inside the heating plate and hold in position by placing under the holding arm.

Tighten the earth screw onto the nanoZero® to ensure that the nanoZero® is grounded and held in position.

Place column directly into the heater recess. Hold in position by placing the holding arm onto the nanoZero®. This requires a replacement metal holding arm (supplied by IonOpticks) to allow an earth connection with the nanoZero®.

The column is now installed in the heater and ready for operation.

Place lid on heater.

Install filter into the CSI housing air inlet.
For Bruker CaptiveSpray source

if using Sonation Column Oven (PRSO-V1 and PRSO-V2)

Retract the column heater from the CaptiveSpray source.

Press the CSI Housing with a small amount of pressure and tighten the screw until finger tight.

Using a gloved finger, block the air inlet and monitor the Fore pressure. The Fore vacuum needs to drop to below 3e-01 mbar within 10 seconds to be considered usable.

If not sealed, try refitting before replacing rubber seals (Refer Bruker CSI troubleshooting guide).

Move the column heater in front of the CaptiveSpray source.

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Holding the nanoZero® with a spanner, tighten the nanoViper fitting finger tight until you reach a firm stop.

Earthing

Place the earth cable clip over the thin section of the nanoZero® and slide over the hexagonal section to make a firm connection. If the clip feels loose, remove the clip, pinch in the clip arms and repeat the process.

Curl the column inside the heating plate and place the nanoZero® and earth clip into the plastic holder.

The column is now installed in the heater and ready for operation.

Closing the heater

Place top plate on heater and secure in position using the press pins. Be careful to avoid pinching the column during this process.

Install filter into the CSI housing air inlet.
For Thermo Scientific Nanospray Flex with Sonation Column Oven (PRSO-V1 and PRSO-V2)

1. Holding the nanoZero® with a spanner, tighten the nanoViper fitting finger tight until you reach a firm stop. Operation of the column with an under tight fitting can result in a fall in performance that cannot be recovered with additional tightening.

2. Preparing the column
   Carefully slide protective sheath backwards towards the nanoZero® fitting to expose the emitter tip. Press the holding clamp on the heater open and place the column into the heater. The column emitter should extend 15-20mm beyond the heating plate.

3. Curl the column inside the heating plate.

4. You will need: 1 x HVCABLE01 for connection of the source high-voltage to the nanoZero® fitting. Connect the HVCABLE01 to the nanoZero as shown.

5. Place top plate on heater and secure in position using the press pins. Be careful to avoid pinching the column during this process.

6. Ensure that the stage is fully retracted in the z-axis before moving the source towards the mass spectrometer.

7. Slide the source into position and adjust the x, y and z axis on the stage to ensure a good emitter position. The emitter should be 3-5mm from the ion transfer capillary.

The column is now installed in the heater and ready for operation.
For Thermo Scientific Nanospray Flex
with 5cm RAPID column housing  (Part No. 5CMNFSH1)

1. Unscrew and remove the existing fastening bolt, marked by the arrow, from the source.

2. Attach the housing using the supplied bolt and nut. Attach the source to the mass spectrometer and plug in voltage connection into the source.

3. Fit the nanoViper into the Aurora column nanoZero® fitting. Expose the emitter tip by sliding the protective sheath towards the nanoZero®.

4. Install Aurora column and nanoViper line into the housing as shown.

5. Place lid on housing.

6. Manoeuvre emitter tip into the desired position using the source stage controls. The emitter should be 3-5mm from the ion transfer capillary.

The column is now installed and ready for operation.
Operation of Aurora Series columns.

Aurora series columns initial operation
Once the column is connected to your UHPLC system and is placed inside the source heater or housing, begin operation using 70% buffer B at a flow rate of 400nl/min for around 10 minutes or until the pressure is stable for several minutes. Voltage should be applied once the mobile phase reaches the emitter tip. It is recommended that at least one gradient is run without sample injection before assessment of column performance using standards.

Standby and Idle conditions
To optimise column lifetime and performance, it is recommended that the instrument continues to run at the desired operating pressure and ideally continues to run blank samples using mobile phase gradients typical of normal operation. Spray voltages should be maintained during these operations.

Extended periods of time at isocratic flow will reduce column performance. Column performance can be recovered by running a blank gradient without sample injection.

Removal from a UHPLC
If possible, do not remove the column from a UHPLC system, however, removal is sometimes required. For the removal procedure, run 80% B for 5min at operating flow rates before reducing flow to 0.002 μl/min for 10min or until the back pressure has stabilised below 10 bar. Set MS system into standby mode. The nanoViper can now be removed from the nanoZero®. For removal of the column for extended periods of time we recommend placing 30μl of methanol in the nanoZero® and screwing in the plug supplied during transport.

Recommended Buffer compositions:
Buffer A 99.9% MilliQ Water, 0.1% formic acid
Buffer B 99.9% Acetonitrile, 0.1% formic acid

Column Volumes
25cm X 75μm 13μl
15cm X 75μm 0.66μl
5cm X 150μm 0.88μl

Column Temperature
The recommended operating temperature for Aurora columns is 40°C. The maximum operating temperature is 60°C.

Recommended Buffer compositions:
Buffer A 99.9% MilliQ Water, 0.1% formic acid
Buffer B 99.9% Acetonitrile, 0.1% formic acid

Column Volumes
25cm X 75μm 13μl
15cm X 75μm 0.66μl
5cm X 150μm 0.88μl

Column Temperature
The recommended operating temperature for Aurora columns is 40°C. The maximum operating temperature is 60°C.

25cm Column
90min gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Composition (% Buffer B)</th>
<th>Flow Rate (μl/min)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.400</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.400</td>
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<td>2</td>
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<td>57</td>
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<td>78</td>
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</tr>
<tr>
<td>101</td>
<td>85</td>
<td>0.400</td>
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Example gradient:

15cm Column
17min gradient

<table>
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<th>Flow Rate (μl/min)</th>
</tr>
</thead>
<tbody>
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<td>0</td>
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<td>0.400</td>
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<tr>
<td>17</td>
<td>30</td>
<td>0.400</td>
</tr>
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<td>85</td>
<td>0.400</td>
</tr>
<tr>
<td>20</td>
<td>85</td>
<td>0.400</td>
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Example gradient:

5cm Column
5min gradient

<table>
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</tr>
</thead>
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<td>2</td>
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<tr>
<td>5</td>
<td>34</td>
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<td>5.5</td>
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<td>6.8</td>
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</tr>
<tr>
<td>7.5</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Example gradient:

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Example data.

The BPC plots shown represent typical results (shown for Hela tryptic digest).

25cm Column, 90min Gradient (200ng injection)

15cm Column, 17min Gradient (200ng injection)

5cm Column, 5min Gradient (100ng injection)
For further information and support visit our website www.ionopticks.com