



GENERATION 4

# NanoShield® Trap Columns User Guide

UHPLC packed trap columns

**Recommended guidelines for optimal setup and operation of NanoShield® Trap Columns:**

NanoShield® 5cm x 100µm C18 traps, 3µm particles 2x nanoZero fittings  
NS1-50100C18-3NZ-3PK

For more  
information, visit  
our Help Centre



## Disclaimer

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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](http://www.ionopticks.com) and the limitations and exclusions of our liability found within those terms. Products should not be used if they appear damaged. 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.

## Regulatory Compliance

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IonOpticks evaluates its products to ensure full compliance with applicable North American and European regulations. IonOpticks products are compliant with the Restriction of Hazardous Substances (RoHS) directive. Changes or modifications to these products not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

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# 1.0 Overview

IonOpticks NanoShield® trap columns set a new performance standard for trap-based proteomic workflows by capturing hydrophilic peptides and delivering near-direct injection performance while providing the essential benefits of in-line sample clean-up, analyte focusing and rapid sample loading.

NanoShield® trap columns are designed to be used with IonOpticks analytical columns to maximise chromatographic efficiency and dramatically enhance performance, providing a best in class solution for peptide and metabolite LC-MS separations.

## 2.0 Product Features

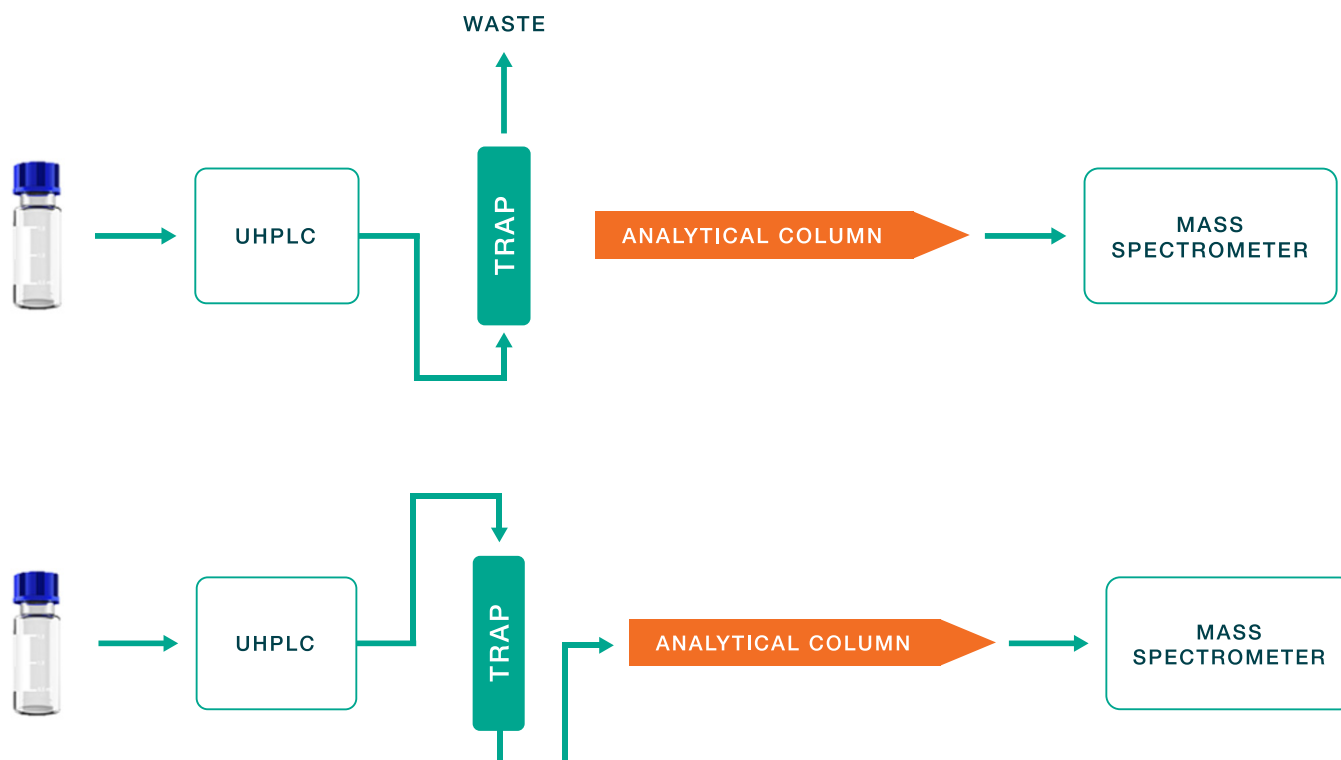
Chromatographic trap columns are essential in proteomic UHPLC–MS workflows, improving analyte focusing and enabling rapid sample loading with in-line sample clean-up to protect analytical columns from blockages and contamination while reducing ion suppression. These benefits extend column lifetime and reduce maintenance, supporting robust, high-throughput, and reproducible proteomic analyses.

Despite these advantages, trap columns traditionally sacrifice peptide and protein identifications compared to direct injection. The reduction in identifications is

often caused by the loss of hydrophilic peptides during sample loading. Early-eluting peptides such as post-translationally modified peptides (e.g., phosphopeptides and glycopeptides), which are typically more hydrophilic, can pass through traditional traps during the sample loading and clean-up steps, creating gaps in proteome coverage and reducing overall identifications. This hydrophilic peptide loss represents a significant compromise compared to the comprehensive analysis achieved with direct injection workflows. NanoShield® C18 trap eliminates this barrier, delivering direct injection-level proteome coverage and identifications as demonstrated above.

### 2.1 Reverse-flush trapping configuration

In **reverse-flush trapping**, analytes are eluted in the opposite direction to loading. This minimises band broadening, improves peak symmetry, and reduces carryover from strongly retained contaminants. Reverse-flush configurations are therefore often preferred in performance-focused workflows, although they do not protect the analytical column from particulates as well as in forward-flush orientations.



## 2.0 Product Features

### 2.2 Feature Considerations of Direct Injection vs. Trapping Methods

Feature / Consideration	Direct Injection	Forward-Flush Trapping	Reverse-Flush Trapping
Sample Loading Speed	Low	High	High
Throughput	Moderate	High	High
In-Line Desalting	No	Yes	Yes
Protection of Analytical Column	Limited	Excellent	High
Retention of Hydrophilic Peptides	High	High	High
Peak Shape	Highest	Moderate	Very High
Sensitivity	Highest	High	Very High
System Complexity	Low	Moderate	Moderate
Ease of Implementation	High	Moderate	Moderate
Typical Applications	Method development, performance-focused, low throughput	Routine, dirty samples, high throughput	Routine, high throughput, performance-focused workflow

#### Performance Guarantee

IonOpticks columns are subjected to rigorous control procedures under the direct eye of our Senior Scientists. All products are covered by our 100% performance guarantee, ensuring any loss of performance due to manufacturing or shipping-related faults will be replaced at no charge. If you have questions or concerns, please contact Support via the Help Centre. Terms and conditions are included with every quotation, but our priority is ensuring users consistently receive a high-quality product, and we welcome feedback at any time.

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## 3.0 Compatibility

IonOpticks NanoShield® trap columns are engineered to pair specifically with Aurora Series analytical columns, using complementary stationary phase to deliver optimal trapping efficiency, peak shape, and chromatographic reproducibility from injection to elution. They also incorporate our nanoZero® technology, providing a user-friendly, plug-and-play connection.

NanoShield® traps (5 cm x 100 µm) are designed for on-valve use with reverse-flush (backward) operation only, and are compatible with several UHPLC systems and workflows.

Product	Reverse / Backward Flush	Forward Flush	
		On Valve	Vented
NanoShield® trap column (5 cm x 100 µm)	✓	✗	✗

Direct UHPLC compatibility below:

Thermo Scientific	Reverse / Backward Flush
UltiMate Series	✓
EASY-nLC 1000/1200	
Vanquish Neo	✓

Bruker	Reverse / Backward Flush
nanoElute	✓
nanoElute 2	✓
proteoElute	✓

Waters	Reverse / Backward Flush
M-Class	✓
nanoAcquity	✓

### Product Specifications

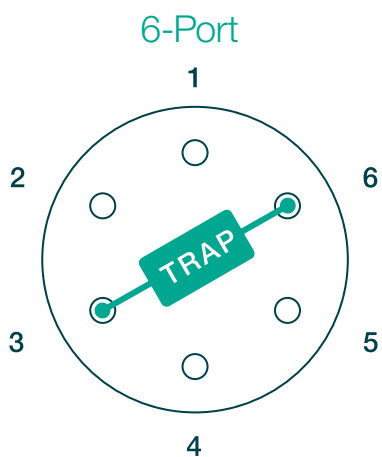
Column format	Trap/guard column
Column type	Reversed-phase
Trap column orientation	Reverse flush
For use with	UHPLC
Length	5 cm
Inner Diameter	100 µm
Pore size	120 Å
Pressure rating	>1700 bar
Particle size	3 µm
pH stability	1–8
Stationary phase	C18
Suggested loading capacity	200 ng
Max loading capacity	1 µg
Suggested loading pressure	< 1000 bar

### Fittings

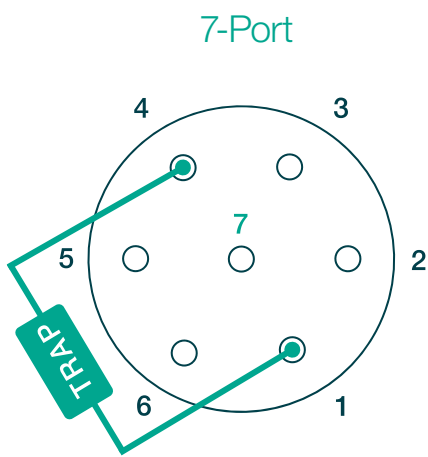
- Thermo Scientific nanoViper
- Waters ZenFit

# 4.0 Reverse Flush Configuration

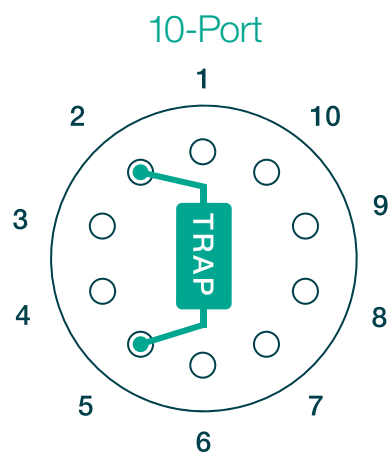
Here are some common valve configurations for 6, 7 and 10 port valves.



- 1 Auto-sampler
- 2 Waste
- 3 Trap
- 4 Gradient pump
- 5 Analytical column
- 6 Trap

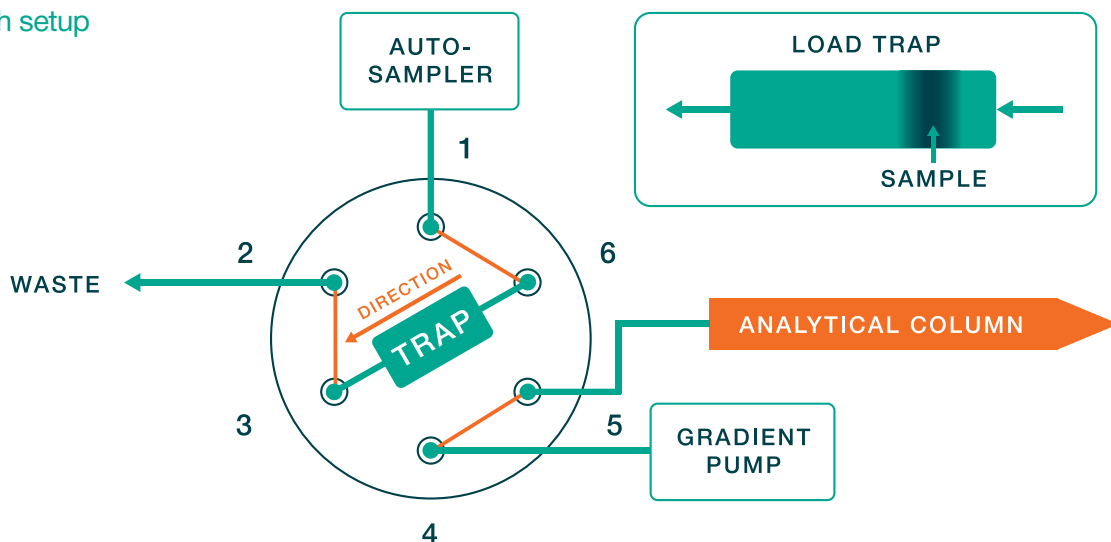


- 1 To right valve
- 2 Waste
- 3 Trap
- 4 Gradient pump
- 5 Analytical column
- 6 Trap
- 7 Pump



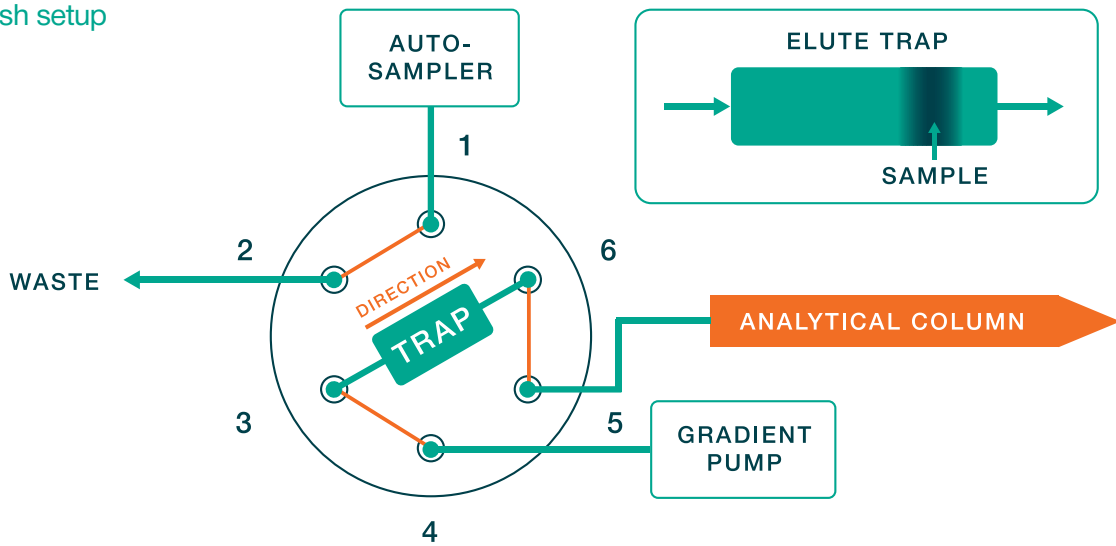
- 1 Waste
- 2 Trap
- 3 Gradient pump
- 4 Analytical column
- 5 Trap
- 6 Auto-sampler
- 7 Waste
- 8 Empty
- 9 Empty
- 10 Empty

## Reversed-flush setup Load (1-6)



# 4.0 Reverse Flush Configuration

## Reversed-flush setup Elute (1-2)

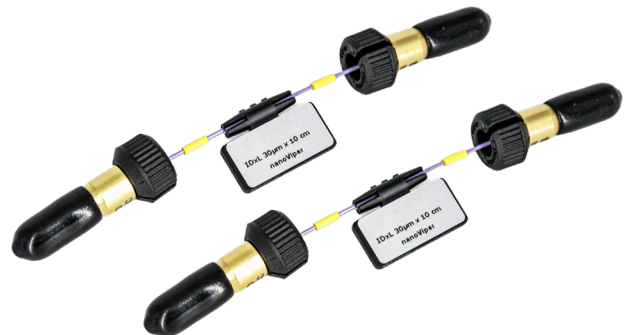


### Recommended for optimal fitting

Two short capillaries are required, such as the Thermo Scientific nanoViper 30 µm ID × 10 cm capillaries.



**For optimal use, please follow these instructions:**  
This product is designed for on-valve use with reverse-flush (backward) operation only. Using it in forward-flush mode may compromise performance



## 4.0 Reverse Flush Configuration

### Installing the NanoShield® Reverse-Flush Trap on your UHPLC

- 1 Reduce flow on your UHPLC to 0.002  $\mu\text{L}/\text{min}$ , until the back pressure stabilises below 10 bar.
- 2 You will need two capillary lines such as 30  $\mu\text{m}$   $\times$  10 cm, or similar. We recommend inspecting both ends of the nanoViper for degradation before use.



Screw one end of each nanoViper line into the appropriate valve port as indicated in your UHPLC user guide.



Remove the shipping plug from the NanoShield® Trap. Connect the free ends of the nanoViper line to each side of the nanoZero union on the trap. NanoShield® Trap (5 cm  $\times$  100  $\mu\text{m}$ ) is non-directional, and can be connected in either direction.



Tighten each nanoViper fitting finger-tight until a firm stop is reached. We recommend applying an additional quarter turn using a 1/4 inch spanner or wrench, as demonstrated. Repeat for the other end of the trap column.



The NanoShield® trap column is now installed and ready to use.

# 5.0 Operation of NanoShield® Trap

## Trap orientation

Can be used for loading and elution in either direction (non-directional)

## Recommended buffer compositions

Buffer A 99.9% MilliQ Water, 0.1% formic acid

Buffer B 99.9% Acetonitrile, 0.1% formic acid

## Trap column temperature

The reverse-flush trap is designed to be operated at ambient temperature. If your LC supports trap heating, the trap can be heated allowing for higher flow-rates.

## Initial operation

Once the trap column is connected to your UHPLC system, begin operation using 80-85% buffer B at a flow rate equivalent to the desired gradient flow rate for around 10 minutes, after which time the pressure should be stable. It is recommended that at least one or two gradients are run without sample injection before assessment of column performance using standards.

## Standby and idle conditions

To optimise trap 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.

## Column equilibration

Before each run the column should be re-equilibrated using a minimum of four column volumes of 100% buffer A. Trap column equilibration should be performed below 1000 bar.

## Column cleaning

The recommended method for cleaning the NanoShield® is to flush with 85% B mobile phase for three column volumes, followed by re-equilibration with 1% B for two column volumes prior to the next injection.

To rejuvenate a used NanoShield®, the column can be flushed with 85% B in both flow directions to help aid the removal of any particulates.

## Trap Column Volume

5cm x 100µm = 0.4uL

## Loading Capacity

- Suggested: <200ng
- Max: 1µg

For optimal results, it is recommended to load <200ng onto the NanoShield®, although the maximum capacity is 1 µg.

Loading capacity is determined by, and should not exceed the capacity of the analytical column. For optimal results, use the lower value between the recommended trap load and the loading capacity of your analytical column. For more information on loading capacity, see Sample Loading:

60 cm x 75 µm	1µg per hour of gradient
25 cm x 150 µm	3µg
25 cm x 75 µm	1µg
15 cm x 150 µm	2µg
15 cm x 75 µm	500ng
8 cm x 150 µm	500ng
8 cm x 75 µm	100ng
5 cm x 150 µm	200ng
5 cm x 75 µm	50ng

## Sample Loading

Depending on your LC system, samples can be loaded using either a pressure limit or a set flow rate. We recommend loading 5–10 times the trap column volume.

## Pressure loading

The recommended loading pressure for NanoShield® is 1000 bar. Pressures above 1000 bar (if supported by your LC system) may cause peak tailing or other undesirable peak characteristics.

## Flow rate loading

If loading by flow rate, we recommend selecting a rate that will not cause your LC system to overpressure. This is typically in the range of 3–15 µL/min.

## Elution, Wash, and Re-equilibration

For elution, use the IonOpticks recommended gradients for your analytical column with the NanoShield® trap. Recommended example gradients for all columns are available [here](#).

Add extra time onto the end of the gradient during the wash and re-equilibration steps to account for any delay introduced by extra volumes due to the NanoShield® trap and capillary lines.



**Note:** When using Aurora Rapid 5x75 columns with NanoShield®, if carry-over is observed, increase wash and equilibration to two to four times the NanoShield® column volume.

## Application notes and Tech notes

Application and technical notes can be found in our Literature Room: <https://ionopticks.com/resource-hub/>






Visit our help centre at: <https://helpcentre.ionopticks.com/portal/en/home> or contact: [support@ionopticks.com](mailto:support@ionopticks.com) for more information.

## 6.0 Removing the NanoShield® Trap on your UHPLC

- 1 When removal is required, reduce the flow rate to 0.002 µl/min for 10 minutes or until the back pressure has stabilised below 10 bar. Set the MS system into standby mode.

**Note: If you intend to store the trap column for extended periods, or re-use it at a later date, start by first flushing the column with 80-50% B prior to removal procedures.**

	<p>Unscrew the nanoViper lines from both ends of the NanoShield® Trap.</p>
	<p>Reinstall the shipping plugs into the nanoZero® unions.</p>
	<p>For removal of the trap column for extended periods of time we recommend placing 30µl of methanol in the nanoZero® and screwing in the plug supplied during transport.</p>



**Note: Removing the solvent line from the nanoZero® union while the system is under high back pressure can damage the stationary phase bed, potentially causing blockages and reduced chromatographic performance in both the trap and analytical column. Always ensure pressure is lowered to safe levels before disconnecting lines.**



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