

HPLC columns

μPAC Neo Plus HPLC columns

Use and care instructions

Product:
Thermo Scientific™ μPAC™ Neo Plus HPLC Columns
(Available in 50, 110, and 5.5 cm formats for nano- and capillary-flow applications)
Pressure limit

Do not exceed the maximum column pressure of 450 bar (6,500 psi). Exceeding this value will cause irreversible damage to the column.

Voltage spacer and grounding

Please use the appropriate voltage spacer for the Thermo Scientific™ EASY-Spray™ Source or Thermo Scientific™ Nanospray Flex™ Ion Source. Each voltage spacer has a connecting pin for attachment of a grounding cable (PN ACC-GRNDC).

Do not use the Vanquish Neo Torque Tool

When connecting or disconnecting with the μPAC Neo Plus HPLC column to or from the Thermo Scientific™ Vanquish™ Neo UHPLC System, do not use the Thermo Scientific™ Vanquish Neo Torque Tool (PN 6250.2110). Fingertight + maximum 1/8th of a turn is sufficient for a dead-volume free connection.

Column connection schematic diagram

A complete μPAC Neo Plus column assembly includes one column, one voltage spacer, one grounding cable, and one ESI emitter (Figure 1). The 5.5 cm μPAC Neo column is recommended for use in an HPLC column compartment. For the 50 cm and 110 cm columns, an external column oven positioned close to the MS ion source is recommended when higher sensitivity is desired.

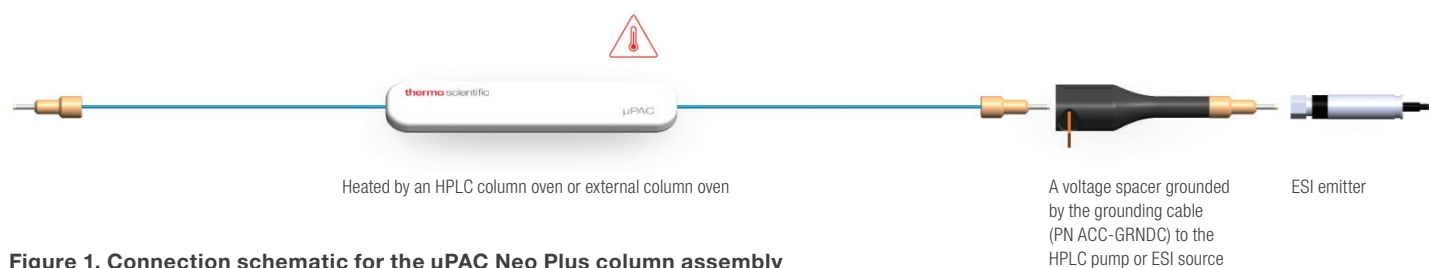


Figure 1. Connection schematic for the μPAC Neo Plus column assembly

Column properties overview

Table 1. Properties overview

Column	Max. column pressure	Max. temperature (°C)	pH stability	Column volume (μL)	Maximum loadability (μg)	Flow rate (μL/min)
μPAC Neo Plus column, 50 cm	450 bar (6,500 psi)	60	2-7	1.5	0.5	0.1-0.75
μPAC Neo Plus column, 110 cm				4.5	2.0	0.1-1.0
μPAC Neo Plus column, 5.5 cm High Throughput				1.5	0.5	0.25-3.0

Installing the column

Inlet

1. The μ PAC Neo Plus column is completely bidirectional with identical lengths of the integrated Thermo Scientific™ nanoViper™ 20 μ m ID capillaries. Either capillary can be used as the inlet or outlet capillary.
2. Attach a stainless-steel Thermo Scientific™ Viper™ union (PN 6040.2304) to the Thermo Scientific™ nanoViper™ Fingertight Fitting on the inlet capillary of the μ PAC Neo Plus column.
3. Connect the transfer line extending from the Vanquish Neo UHPLC system autosampler valve to the Viper union attached to the column.
4. With the black knurled nut, carefully turn the fitting until fingertight. Do not overtighten, maximum 1/8th of a turn past fingertight.
5. Remove the black knurled nut after tightening.
6. To assess the connections, apply the desired flow rate, wait one column volume (see Table 1) and verify that the pressure is within the expected values at 50 °C listed in Table 2.
7. When installing the μ PAC Neo Plus column in the Vanquish Neo column compartment, use the column kit (P/N 6722.0290, included in the Vanquish Neo column compartment ship kit) together with the μ PAC column patches (Figure 2).

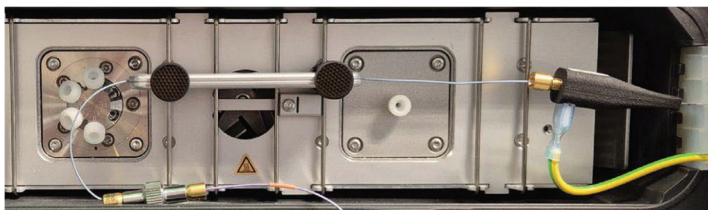


Figure 2. Installation of the μ PAC Neo Plus column in the column oven using column clips and patches

Outlet

When connecting the μ PAC Neo Plus column to the mass spectrometer, always ensure a grounded connection between the μ PAC column and the HV power supply for proper chromatographic behavior and optimal results (detailed in the Grounding chapter).

To establish a grounded connection between the column and the ESI emitter, connect the grounding cable (PN ACC-GRNDC) to the voltage spacer. Different voltage spacers are available depending on the set-up and position of the column. Refer to the recommended voltage spacers (Tables 3 and 4) for EASY-Spray and Nanospray Flex ion sources (Figures 3 and 4).

Detailed installation instructions and safety precautions of each respective voltage spacer are provided with the accessory.

1. Connect the outlet of the column to the appropriate voltage spacer. Do not use a torque tool as this may damage the nanoViper fitting, fingertight is sufficient.
2. Connect the voltage spacer to the selected ESI emitter. Do not use a torque tool as this may damage the nanoViper fitting, fingertight is sufficient.
3. Apply a recommended flow rate between 100 nL/min and 3,000 nL/min (depending on column type and voltage spacer selected). Droplets should appear at the tip of the emitter.
4. Ensure that the pressure has not increased by more than 10% compared to the value recorded earlier without the voltage spacer and emitter (see Table 1).
5. Apply ESI voltage in the MS control software and monitor the spray.
6. With the XYZ manipulator (Nanospray Flex ion source) or a hex key (EASY-Spray ion source) position the tip of the emitter almost directly in-line with the ion transfer tube of the mass spectrometer.

Note: if the columns are used in the Tandem Direct Injection workflow of the Vanquish Neo UHPLC system where the outlet is connected to a switching valve, no voltage spacer (and grounding cable) is needed as the switching valve is already grounded.

Table 2. Settings for assessing the connection between the injection valve and μ PAC Neo Plus HPLC column without an emitter connected

μ PAC Neo Plus column Part No.	COL-UPAC050NAN	COL-UPAC110NAN	COL-UPAC005CAP
Flow rate (nL/min)	Pressure (bar) at 50 °C		
300	100-150	100-150	25-75
500	175-250	175-250	50-100
750	275-375	275-375	75-125
1,500	-	-	100-175
2,500	-	-	225-300

Table 3. μ PAC Neo Plus connection accessory selection for low-flow ESI emitters

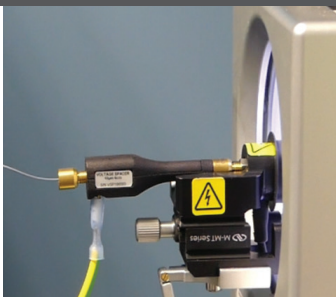
μ PAC Neo Plus column Part No.	Emitter Part No.	Voltage spacer Part No.	Grounding cable Part No.
COL-UPAC050NAN	ES993 (EASY-Spray source) or ES542 + 00109-02-00055 (Nanospray Flex ion source)	ACC-VSP2050 (Vanquish Neo Thermostated Column Compartment) or ACC-VSP1006 (external column oven)	ACC-GRNDC
COL-UPAC110NAN			
COL-UPAC005CAP	ES994 (EASY-Spray source) or ES542 + 00109-02-00055 (Nanospray Flex ion source)	ACC-VSP2050 (Vanquish Neo Thermostated Column Compartment) or ACC-VSP2006 (external column oven)	

Table 4. μ PAC Neo Plus connection accessory selection for 3rd party 360 μ m OD emitters

μ PAC Neo Plus column Part No.	Emitter Part No.	Voltage spacer Part No.	Grounding cable
COL-UPAC050NAN	Any 360 μ m OD emitter (Nanospray Flex ion source)	ACC-NANOFIC10 (external column oven)	ACC-GRNDC
COL-UPAC110NAN			
COL-UPAC005CAP		ACC-CAPFIC20 (external column oven)	

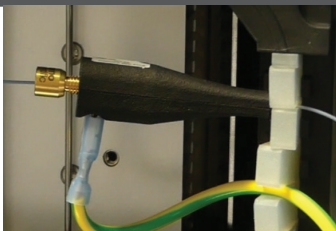
Outlet connected to the EASY-Spray ion source without on-source column heater

When the column is heated by an external column oven, connect the nanoViper fitting on the column outlet capillary to the short voltage spacer for EASY-Spray source for nano-flow (PN ACC-VSP1006) or for capillary-flow (PN ACC-VSP2006) applications.



Outlet connected to either EASY-Spray or Nanospray Flex ion source when column is positioned in the LC column compartment

Connect the nanoViper fitting on the outlet capillary to the long voltage spacer (PN ACC-VSP2050) for EASY-Spray ion source or Nanospray Flex ion sources.



Long voltage spacer source connected to ESI emitters

Connect the EASY-Spray Bullet emitter for nano-flow (PN ES993) or for capillary-flow (PN ES994) applications into the EASY-Spray ion source.



Connect the Thermo Scientific™ Stainless Steel Emitter (PN ES542), then place it onto the Nanospray Flex ion source.

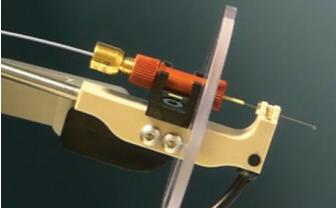


Figure 3. μ PAC Neo Plus column outlet connection to EASY-Spray source or Nanospray Flex on source compatible voltage spacers.

Connection of voltage spacer outlet to emitter with integrated liquid junction or reducing union.

Outlet connected to the Nanospray Flex ion source with on-source column heater

Connect the nanoViper fitting on the outlet capillary to the inlet of the Flex IonConnect for nano-flow (PN ACC-NANOFIC10) or for capillary-flow (PN ACC-CAPFIC20) applications.



Connect 3rd party fused silica emitter to the outlet of the Flex Ion Connect and then place it onto the Nanospray Flex ion source.

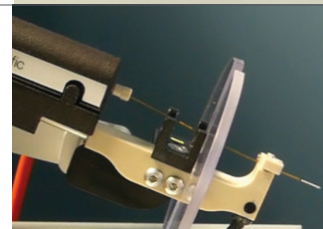


Figure 4. μ PAC Neo Plus column outlet connection to Nanospray Flex ion source compatible voltage spacer. Mounted with a 360 μ m OD emitter on a NanoSpray flex source.

Grounding

The grounding cable (PN ACC-GRNDC) features a fork (or spade) terminal on one end and a female terminal on the other.

All voltage spacer accessories are equipped with a clip that fits the female terminal of the grounding cable.

1. Slide the female terminal over the grounding clip. Apply a little pressure to ensure the terminal slides onto the grounding clip but take care as the clip can have sharp edges.
2. Connect the fork terminal to a suitable grounding point on the mass spectrometer, ESI source or the HPLC instrument. Refer to the use and care instructions for μ PAC Neo Plus voltage spacer for EASY-Spray source.

Recommendations for column specification and method settings

- Please follow the recommended column specifications and method settings when operating the Vanquish Neo UHPLC system, as outlined in Table 5 (Specifications), Table 6 (Direct injection), and Table 7 (Trap-and-elute).
- When performing trap-and-elute with μ PAC trapping columns, ensure that the Thermo Scientific™ Vanquish™ Neo System driver package 1.6 or higher is installed to prevent trap column damage during cleaning cycles

Table 5. Separation column specification settings in the Vanquish Neo system interface — A04 script

Description	50 cm μ PAC Neo Plus HPLC column (PN COL-UPAC050NAN)	110 cm μ PAC Neo Plus HPLC column (PN COL-UPAC110NAN)	5.5 cm μ PAC Neo Plus HPLC column (PN COL-UPAC005CAP)
Diameter (μ m)	75	75	75
Length (cm)	50	150	50
Max. pressure (bar)	450	450	450
Max. flow* (μ L/min)	1	1	3
Max. temperature ($^{\circ}$ C)	60	60	60
Max. pressure change up (bar/min)	1000	1000	1000
Max. pressure change down (bar/min)	1000	1000	1000

*The maximum flow rate must not exceed the recommended limit to ensure robust and stable column operation. Exceeding this limit may reduce column lifetime.

Table 6. Recommended settings for direct injection

Category	Parameter	
Sample loading	Fast loading	Enabled
	Mode	Pressure control
	Flow	/
	Pressure	450 bar
	Loading volume	1.5 μ L
Column equilibration	Fast equilibration	Enabled
	Mode	Pressure control
	Flow	/
	Pressure	450 bar
	Equilibration factor	1
Temperature	Column compartment temperature	50 $^{\circ}$ C
	Autosampler temperature	7 $^{\circ}$ C

Table 7. Recommended settings for trap-and-elute with double nanoViper μ PAC trap

Category	Parameter	
Sample loading	Mode	Flow control
	Flow	20 μ L/min
	Loading volume	1.5 μ L
Column equilibration	Fast equilibration	Enabled
	Mode	Pressure control
	Flow	/
	Pressure	450 bar
	Equilibration factor	1.0
Trap column	Fast wash and equilibration	Enabled
	Wash factor	Automatic
	Equilibration factor	Automatic
	Mode	Combined control
	Flow	40 μ L/min
	Pressure	400 bar
	Trap flush direction	Backward
Temperature	Column compartment temperature	50 $^{\circ}$ C
	Autosampler temperature	7 $^{\circ}$ C
	Trap cartridge	Room temperature (ca. 23 $^{\circ}$ C)

Application recommendation

Preparation for use

1. μ PAC Neo Plus HPLC columns are prefilled with 70% acetonitrile.
2. Before use, flush the column with 70% acetonitrile or methanol and equilibrate the column with at least one column volume (see Table 1) of the desired starting solvent.

Mobile phase and sample

1. Use only fresh, degassed, and LC-MS grade mobile phases that are compatible with RP-LC.
2. Switch only between mutually miscible mobile phases.
3. Column lifespan is greatly affected by sample cleanliness. Use samples which are free from particulates. Filter if necessary, using a 0.5 μ m cut-off filter.
4. Typical solvents: acetonitrile (ACN), methanol (MeOH), isopropanol (IPA), trifluoroacetic acid (TFA), formic acid (FA), and water (H_2O).

Improper grounding

Improper grounding would result in peak broadening and low LCMS performance

1. The column can be restored by correctly grounding the column using the grounding clip provided on the voltage spacer.
2. Apply isocratic flow (or run several blank injections).
3. Charging effects should dissipate within approximately 4 hours and column performance will be completely restored.

Technical support

For support with Thermo Scientific™ chromatography consumables, visit our online support portal for fast, self-service access to product documentation, certificates, and troubleshooting resources. You can also submit a support ticket to connect directly with a technical expert for more complex inquiries. Go to thermofisher.com/chromatographysupport for more information.

 Learn more at thermofisher.com/lowflowhplccolumns

Column care

- To prevent damage, handle μ PAC Neo Plus columns, capillaries and accessories with care.
- Never remove the nanoViper PEEK tubing extending from the aluminium jacket, nor cut the PEEK tubing. Do not open the housing of the column. This will damage the μ PAC Neo column and prevent further use.

Column storage

- μ PAC Neo columns can be stored for short periods in most mobile phases.
- For prolonged storage, it is recommended that the μ PAC Neo Plus column is flushed with 5-10 column volumes of a mobile phase with at least 70% ACN in water.
- If the μ PAC Neo Plus column has been used with buffered mobile phases, remove the buffer by flushing with 5-10 column volumes of a mobile phase containing 50% ACN or MeOH in water and then flush with the storage mobile phase.
- Avoid storage of the μ PAC Neo Plus column in mobile phases containing TFA.

Column regeneration

- If the backpressure increases above 150% of the original value, reverse the flow direction of the μ PAC Neo Plus column and flush with 5-10 column volumes of mobile phase. This should return the μ PAC column to the original backpressure.
- Alternatively, flush the column with Thermo Scientific™ ChromaCare™ LC-MS Instrument Flush Solution (PN T111101000) with added 0.1% TFA, followed by an overnight flush with mobile phase at 50 - 100 nL/min.

Disclaimer and contact

Warranty of the column extends up to 30 days after the purchase of the product.