HPLC columns

µPAC Neo HPLC columns

Use and care instructions

Products:
Thermo Scientific™ µPAC™ Neo HPLC Columns (50 cm, 110 cm, 50 cm Low Load, and 5.5 cm High Throughput column formats)

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.

Installing the µPATCH column holder

1. Attach the column clips to the inside of the column compartment or column oven.
2. Using the two slots at the top and bottom of the Thermo Scientific™ µPATCH™ Column Holder, install the µPATCH column holder as shown in Figure 1.

Note: As the µPAC Neo column is asymmetrical, ensure the orientation of the column is correct, with the outlet extending towards the source of the mass spectrometer (the longer capillary is recommended as the outlet from the column).

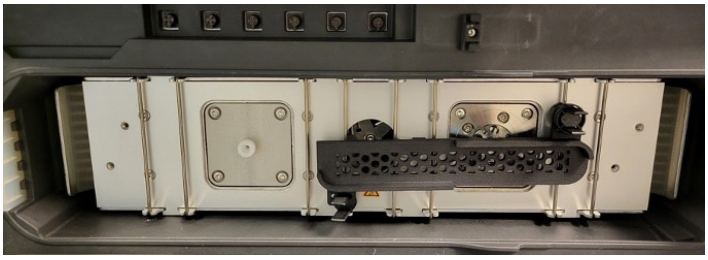


Figure 1: µPATCH column holder installed in either the column compartment or the column oven.

Installing the column

Inlet

1. The µPAC Neo column is completely bidirectional but the lengths of the integrated Thermo Scientific™ nanoViper™ 20 µm ID Capillaries are different. The short capillary is recommended as the inlet to the column.
2. Attach a stainless-steel Thermo Scientific™ Viper™ union (P/N **6040.2304**) to the Thermo Scientific™ nanoViper™ fitting on the inlet capillary of the µPAC Neo column.
3. Connect the transfer line extending from the Thermo Scientific™ 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 1/8 of a turn after 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 2) and verify that the pressure is within the expectations at 50 °C listed in Table 1.

Outlet

1. The long capillary is recommended and preferred as the outlet from the column.
2. When using a Thermo Scientific™ EASY-Spray™ Ion Source, connect the nanoViper fitting on the outlet capillary to either a Thermo Scientific™ EASY-Spray™ Nano or Capillary Bullet Emitter (P/N **ES993**) or P/N **ES994**, respectively). Insert the EASY-Spray emitter into the EASY-Spray ion source.

Table 1. Settings to assess the connection between the injection valve and µPAC Neo HPLC column

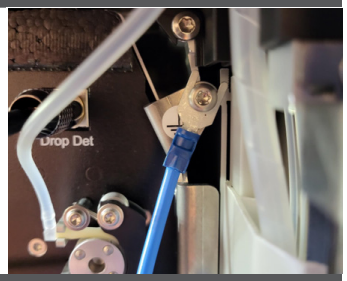
µPAC Neo column	50 cm	110 cm	50 cm Low Load	High Throughput
Flow rate (nL/min)	Pressure (bar)			
300	100-150	100-150	110-160	25-75
500	175-225	175-225	200-250	50-100
750	300-350	300-350	310-360	100-150
1,500	–	–	–	200-250
2,500	–	–	–	325-375

Grounding

1. In the bottom of the shipping box under the foam supporting the µPAC Neo column is a grounding cable. This grounding cable has a fork (or spade) terminal on one end and a female terminal on the other.
2. All µPAC Neo columns have clip that fits the female terminal of the grounding cable.
3. Slide the terminal over the grounding clip. As a little pressure must be applied to ensure the terminal slides onto the grounding clip, take care with this step as the clip can have sharp edges.
4. Connect the fork terminal to a suitable grounding point on the mass spectrometer or the HPLC instrument.

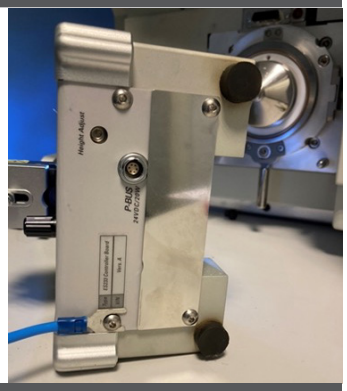
LC system

- Connect the fork to the LC chassis



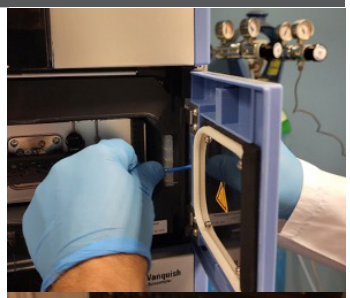
EASY-Spray ion source

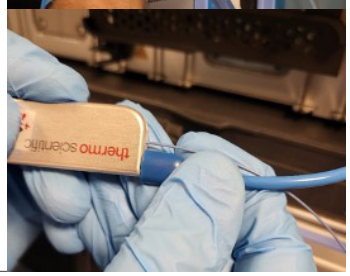
- Use a 2.5 mm Allen key to loosen the screw on the bottom of the EASY-Spray source
- Slide the fork underneath the screw head
- Tighten this assembly with the Allen key



µPAC Neo column grounding point

- Feed the grounding cable with the female terminal through the slit between the door of the column compartment.
- Slide the terminal over the grounding clip. As a little pressure must be applied to ensure the terminal slides onto the grounding clip, take care with this step as the clip can have sharp edges.





Column use

Preparation for use

1. µPAC Neo HPLC columns are filled 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 2) 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. Cleanliness of the sample greatly affects the column life.
4. Use (clean) samples which are free from particulates. Filter if necessary, using a 0.5 µm cut-off filter.

Typical solvents: acetonitrile (ACN), methanol (MeOH), isopropanol (IPA), trifluoroacetic acid (TFA), formic acid (FA)

Column care

- To prevent damage, handle µPAC Neo 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 µPAC Neo column and prevent further use.

Column storage

- µPAC Neo columns can be stored for short periods in most mobile phases
- For prolonged storage, it is recommended that the µPAC Neo column is flushed with 5-10 column volumes of a mobile phase with at least 70% ACN in water
- If the µPAC Neo 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 µPAC Neo column in TFA containing mobile phase

Column regeneration

- If the backpressure increases above 150% of the original value, reverse the flow direction of the µPAC Neo column and flush with 5-10 column volumes of mobile phase. This should return the µPAC column to the original backpressure.
- Alternatively flush the column with 25% IPA, 25% ACN, 25% MeOH, 25% H_2O and 0.1% TFA (high viscosity) and flush overnight at 50 – 100 nL/min.

Improper grounding

1. The column can be restored by grounding the column correctly at the grounding clip.
2. Apply isocratic flow (or inject some blanks).
3. After approximately 4 hours, charging effects should be gone, and the column performance completely restored.

Column properties overview

Table 2. Properties overview

Column	Max. column pressure	Max. temp (°C)	pH stability	Column volume (µL)	Loadability (µg protein)	Flow rate (µL/min)
µPAC Neo column, 50 cm	450 bar 6,500 psi	60	2-7	1.5	0.5	0.1-0.75
µPAC Neo column, 110 cm	450 bar 6,500 psi	60	2-7	4.5	2	0.1-0.75
µPAC Neo column, 50 cm Low Load	450 bar 6,500 psi	60	2-7	1.5	<0.01	0.1-0.75
µPAC Neo column, High Throughput	450 bar 6,500 psi	60	2-7	1.5	0.5	0.25-2.5

Disclaimer and contact

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

Learn more at **thermofisher.com/chromatographyconsumables** or contact us

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