

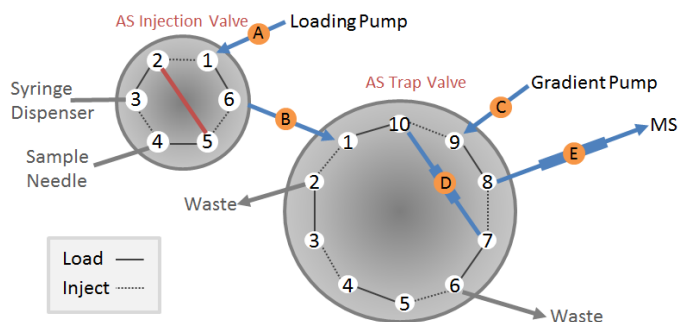
# Configuring the NanoLC™ 425 System for Both Nanoflow and Microflow LC-MS Workflows

## Brief Instructions and Consumables List

Omics research often requires a broad range of workflows, from high sensitivity discovery to high throughput targeted quantitation. The ability to couple a single liquid chromatography (LC) system, that is both flexible and provides high performance and interchangeable nano and microflow LC, to a mass spectrometry (MS) system offers convenience to perform wide variety of applications with increased productivity, and cost benefits.

To set up the system for rapid switching between nanoflow and microflow workflows, the system can be configured as follows:

- With the dual gradient system, one channel is configured with the nanoflow module (0.1 – 1  $\mu\text{L}$  / min).
  - Use a 25  $\mu\text{m}$  ID tubing to connect this channel to the AS trap valve when performing nanoflow (See Table 2 for part numbers, Figure 1, green).
- The second channel is configured with the microflow module (1 – 10  $\mu\text{L}$  / min).
  - Use a 50  $\mu\text{m}$  ID tubing to connect this channel to the AS trap valve when performing microflow (See Table 1 for part numbers, Figure 1 blue).



**Figure 1. Plumbing Diagram for Switching the NanoLC™ 425 System Between NanoFlow and Microflow.** The basic plumbing for the pumps and the two autosampler valves is shown. The setup for the AS Injection valve remains the same for both nanoflow and microflow. When switching between nanoflow and microflow, connect the correct gradient channel to the AS trap valve to port 9, change the trap column that is connected between ports 7 and 10, then connect the column using the correct nanoflow/microflow tubing out of port 8 and then to the MS source.

- The isocratic Loading pump is configured with the Loading pump flow module
  - Use a 50  $\mu\text{m}$  ID tubing to connect the Loading pump to the AS injection valve, and then the injection valve to the Trap valve (See Table 1 for lengths and part numbers).
- The plumbing on the autosampler injection valve (where the Loading pump is used to load the sample from the sample loop onto the trap column) does not need to change when switching between nanoflow and microflow rates.
- Setting up the system for nanoflow LC:
  - Connect the nanoflow gradient channel to the correct port in the autosampler trap valve (Figure 1, green).
  - Connect the nanoflow trap between ports 7 and 10
  - Connect the nanoflow column to port 8, then connect the column to the MS source
- To switch from nanoflow to microflow LC:
  - Connect the microflow gradient channel to the correct port in the autosampler trap valve (Figure 1, green).
  - Connect the microflow trap between ports 7 and 10
  - Connect the microflow column to port 8, then connect the column to the MS source
- If the Gradient Channel has not been used for a few weeks, perform 5 purge cycles, a short flush of the channel, then reinitialize the pressure transducers before connecting the tubing to the AS Trap valve.

**Table 1. Parts List for Configuring the NanoLC™ 425 System for Either Nanoflow or Microflow Chromatography.** This table provides the part numbers for all the consumables needed for either flow regime. Column 1 describes how each consumable is used or where it is located on Figure 1. Note the parts that are marked in grey are the same for both flow regimes and therefore do not need to be changed when switching modes.

Description or Figure 1 Item	Nanoflow LC Configuration	Microflow LC Configuration
Flow Module	<i>Ekspert NanoLC 400, Nano flow module for 0.1 – 1 µL / min (P/N 5018236)</i>	<i>Ekspert NanoLC 400, Low micro flow module for 1-10 µL/min (P/N 5018237)</i>
<b>A – Tubing - Load pump to AS injection valve</b>	<i>Tubing, 50 µm i.d., 1/32 inch o.d., 75 cm PEEKsil (natural) (P/N 205-00049)</i>	
<b>B – Tubing - AS injection valve to AS trap valve</b>	<i>Tubing, 50 µm i.d., 1/32 inch o.d., 30 cm PEEKsil (P/N 205-00040)</i>	
<b>C – Tubing - Gradient pump to AS trap valve</b>	<i>Tubing, 20 µm i.d., 1/32 inch o.d., 75 cm PEEKsil (P/N 205-00048)</i>	<i>Tubing, 50 µm i.d., 1/32 inch o.d. 75 cm PEEKsil (natural) (P/N 205-00049)</i>
<b>D - Trap</b>	<i>ChromXP NanoLC Trap column 350 µm id x 0.5 mm, ChromXP C18 3 µm 120Å (2/pkg) (P/N 5016752)  (Tubing Integrated)</i>	<i>Trap Cartridge holder with 1/32in peek fittings (P/N 5027467)  0.3 mm trap cartridges, ChromXP C18CL 5µm 120A 5/pk (P/N 5028897)  Tubing, 50 µm i.d., 1/32 inch o.d., 10 cm PEEKsil (P/N 205-00069, order 2)  5 PK, Fitting, 1/32 in PEEKSil to 1/16 in 10-32 Port (P/N 5019821)  5 PK, Ferrule, 1/32 in PEEKSil to 1/16 in 10-32 Port (P/N 5019820)</i>
<b>E - Column</b>	<i>ChromXP NanoLC column 75 µm id x 15 cm, ChromXP C18 3µm 120Å (P/N 805-00120)  (Tubing Integrated)</i>	<i>Tubing, 50 µm i.d., 1/32 inch o.d. 75 cm PEEKsil (natural) (P/N 205-00049)  Column, 3µm, ChromXP C18CL, 120A, 150 x 0.3mm (P/N 5022436)  Tubing, 25 µm i.d., 1/32 inch o.d., 10 cm PEEKsil (P/N 205- 00091)</i>
Spray Tip	<i>Nanoflow spray tip 360/20 µm ID/OD; 10 µm tip; 20/pkg (P/N 910-00052)</i>	<i>25 Micron ESI Electrode (P/N 5028467)</i>
PEEK nut and ferrule	<i>1/32 inch PEEK nut with glass-filled PEEK ferrule (10/pkg) (P/N 5019621)</i>	
PEEK Sleeves	<i>1/32" sleeve for 365 µm OD fused silica capillary 10/pkg (P/N 910-00088)</i>	N/A

**Table 2. Parts List for Other Consumables Required During LC-MS Operation.**

Part Number	Item	Description
200-00320	Tubing, FEP, 1/32 OK 5ft roll	Waste tubing for AS trap valve
910-00082	PEEKsil sample loop 10 $\mu$ l, 1/32" OD	Injection loop for AS valve
910-00104	11 mm PP 250 $\mu$ l vial (100/pkg)	Vials for proteomic analysis
910-00103	11 mm snap cap with slit septum (100/pkg)	Slit septum lids for vials
0950.325	PEEKsil 3.6 $\mu$ L sample needle NanoLC 400 AS	AS Sample Needle
205-00069	Tubing, 50 $\mu$ m i.d., 1/32 inch o.d., 10 cm PEEKsil	<b>Optional:</b> Higher ID tubing for robustness, for post column connection to source
5028466	50 Micron ESI Electrode	<b>Optional:</b> Higher ID electrode for robustness, to adapt DuoSpray / Turbo V Source for microflow.

## References

1. Achieving Very High Reproducibility for Quantitative Proteomics with Nanoflow LC/MS - NanoLC™ 400 Series System. SCIEX Technical Note RUO-MKT-02-5755-A.
2. SWATH Performance Kit - <https://sciex.com/products/standards-and-reagents/swath-acquisition-performance-kit>

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