

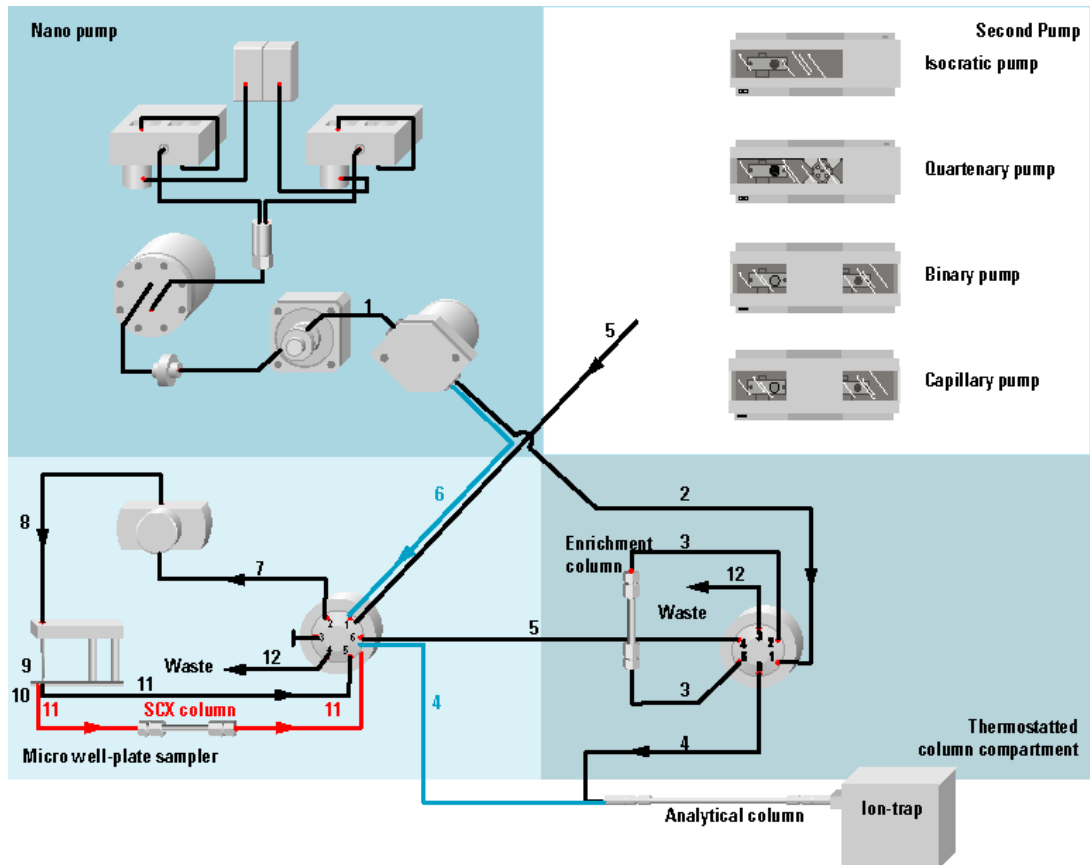


Agilent Nanoflow Proteomics Solution

Quick Reference Guide

The diagram below provides an overview of all capillaries needed for complete setup of an Agilent Nanoflow Proteomics Solution. You can use any Agilent 1200 Infinity Series pump as second pump.

For details on each of the capillaries see [Table 1](#) on page 4.



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Tips for Successful Operation of Nanoflow Proteomics Solution

System

- For direct injection place the Well-plate Sampler close to the Ion Trap.
- Note system pressure of your newly installed system under typical conditions (40-50 bar at 300 nl/min of water with a 50x0.075 mm, 3.5 µm column).
- Check for plugged column capillaries if pressure increases more than 30 %.
- Allow enough trapping/injection time for the sample to be transferred to the trapping or analytical column.
- Replace the 100 µm seat capillary with the 75 µm seat capillary (G1375-87316).
- For **2DLC** install the 40 µl loop capillary (G1377-87300).

Capillaries

- Wash both ends with organic solvent and flush before connecting new capillaries to other components.
- Always install or retighten without flow.
- Use pH below 9.
- Compare capillary pressure drop according to [Table 1](#), replace it if you have more than 30 % deviation.
- Avoid gaps within fittings.
- Do not overtighten, trap (in module doors) or bend with radius smaller than 4 cm.
- Inspect suspicious capillary (milky surface) under microscope.
- Replace capillaries with permanent sharp bends.

Pump/Degasser

- Use primary flow rate for low solvent consumption.
- After changing solvents, purge each channel for 4 min.
- After sitting idle, set composition to 50 % ACN and pump until pressure ripple is less than 3 %.
- Check pressure drop of solvent filter in front of the EMPV once a month.
- It is highly recommended that the nano pump always remains running. However, if the system sits idle for a day or longer, flush each channel for a few minutes.
- System backpressure should be higher than 20 bar.
- Irregular flow/pressure fluctuations indicate partially blocked capillaries.
- Regular fluctuations indicate air within the high pressure path.
- Rotate EMPV valve once while under flow to remove dirt from the valve seat.
- Fast composition change is not used for Nanoflow proteomics solution.
- Never run without solvent inlet filters.
- Use glass bottled solvents.
- Use clean solvent bottles and solvent.

Well-plate Sampler (WPS)

- Cool sample.
- Use needle wash.
- Prime and verify wash pump once a week.
- Ensure comparable pressure drop in a mainpass and bypass once a week.
- Set **bottom sensing** ON with a 0.9 mm offset for the plastic micro insert vials.
- For direct injection use bypass mode, allowing 3-6 min (300 nl/min) sample transfer between WPS and column.
- Check alignment once a month.

Nanoelectrospray

- Cut the needle before installation.
- Horizontal positioning of needle tip should be approx. 3 mm from counter electrode, or such that both are seen at opposite ends in the microscope's field of view. It should also be in line with the counter- electrode and the capillary cap. Adjust the vertical positioning so that the distance between the tip and the counter electrode /capillary cap axis of symmetry is more than the smaller diameter beveled edge of the counter electrode and less than the outer diameter beveled edge.
- Set voltage between 1800 and 2000 volts to generate a capillary current of approximately 100 nA.
- It is recommended that the LC flow always remain **ON**, which will keep the needle from plugging because the solvent is never allowed to remain static, leading to evaporation and possible precipitation. However, if the LC flow must be stopped temporarily, remove the needle assembly from the source to a cooler location.
- Gently push the needle's blunt end through the needle nut then through the conductive ferrule until it protrudes a couple of millimeters. Rinse the blunt tip with isopropanol. Use a flat, clean surface to push the blunt tip back through the ferrule.

Acquisition method settings




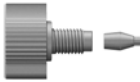
- For analyzing a protein digest sample in the **Direct**, **Enrichment**, or **2DLC** modes, start acquisition using the corresponding methods provided to you by your Agilent representative and/or contained on the LC/MSD Trap Control & Data Analysis software CD-ROM.

For more information on your Agilent Nanoflow Proteomics Solution please check the *Nanoflow Proteomics Solution Getting Started Guide* (G4000-90020), the *Nanospray Users Guide* (G1982-90005) and the *Nano Pump User Manual* (G2226-90010).

Table 1 Capillaries of the Agilent Nanoflow Proteomics Solution

Item	Fitting type*	Material	Diameter (µm)	Length (mm)	Volume (nl)	Pr. drop (bar) for 2 µl/min H ₂ O	Part number
1	B/D	PFS	25	220	108	9	G1375-87321
2 (Direct/Enrichment)	D/C	PFS	25	350	172	15	G1375-87322
3	C/D	PFS	25	100	49	4	G1375-87320
4 (Direct/Enrichment)	C/D	PFS	25	700	344	28	G1375-87324
5	B/C	PFS	75	650	2872	0.6	G1375-87327
6 (Direct only)	D/C	PFS	25	550	270	23	G1375-87323
7	C/B	PFS	100	200	1571		G1375-87312
8 (8 µl Loop Capillary)	B/D	PFS	100	1100	8639		G1375-87315
8 (40 µl Loop Capillary)	B/D	SST	250	2144	105000		G1377-87300
9 (Needle)	B/-	SST	130	94.5	1254		G1377-87201
10 (Needle seat)	-/B	SST			74		G1377-87101
11 (Enrichment / 2DLC)	B/C	PFS	75	150	663	0.15	G1375-87316
12 (Waste tube)	C/-			2000			G1375-87326
Restriction capillary	C/B	FS	25	8000	3927	280	G2226-67300

Table 2 Fitting and ferrules

Fitting type	Name	Description	Conditioning	Part Number
B 	Lite Touch	4/16" SST fitting	10/pk	5063-6593
B 	Lite Touch	1/32" SST ferrule and lock ring	10/pk	5065-4423
C 	Rheodyne	M4 PEEK fitting	6 fitting/2 plug	5065-4410
D 	Finger Tight	Double winged nuts and 1/32" ferrules	10/pk	5065-4422

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Part Number
G2228-90011

Edition 06/10
Printed in Germany



G2228-90011