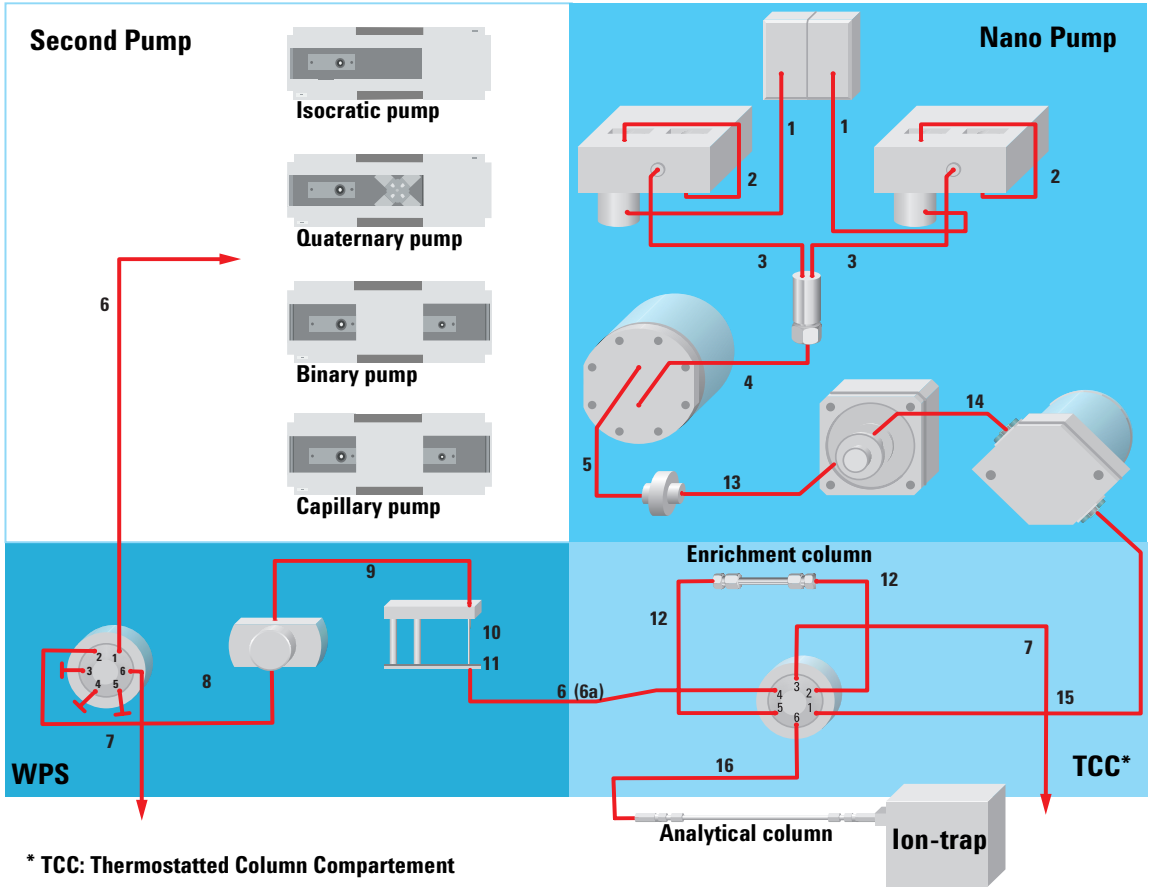
Agilent Nanoflow Proteomics Solution

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 1100 series pump as second pump.

For details on each of the capillaries see Table 1 on page 4.



* TCC: Thermostatted Column Compartement

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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 new 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.

Capillaries

- Wash both ends with organic solvent and flush before connecting new capillaries to other components.
- Always install or retighten without flow.
- Use pH lower than 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 clean solvent bottles and solvent.
- 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.
- After sitting 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.

Well-plate sampler (WPS)

- Cool sample.
- Use needle wash.
- Prime and verify wash pump once a week.
- Check alignment once a month.
- Ensure comparable pressure drop in a mainpass and bypass once a week.
- Use **bottom sensing** for minimum sample solvent.
- For direct injection use bypass mode, allowing 3-6 min (300 nl/min) sample transfer between WPS and column.

Ion Trap

- Cut and rinse sprayer needle before installation.
- Gently push the needle through the conductive ferrule until it touches the filter screen of the column and pull it back a little bit before handtighten.
- New needles sometimes need some positioning back and forwards to the plate until a good spray is obtained.
- Set voltage to about 1400 V to generate a current of a few hundred nA.
- Do not use highly conductive sample solvents (e.g. 1 %TFA) to avoid arcs (needle damage).
- Do not leave the needle close to the plate without flow, e.g. after a sequence use a method with low dry temperature.

For more information on your Agilent Nanoflow Proteomics Solution please check the *Nanoflow Proteomics Solution Getting Started Guide* (G4000-90020) and the *Nano Pump User Manual* (G2226-90000).

Table 1 **Capillaries of the Agilent Nanoflow Proteomics Solution**

Item	Fitting type*	Material	Diameter (µm)	Length (mm)	Volume (µl)	P. drop (bar) for 1 µl/min H2O	Part number
1	A/A	SST					G1311-67304
2	A/A	SST					G1312-67300
3	A/A	SST					G1312-67302
4	A/A	SST					G1312-67304
5	A/A	SST	250	130	6.381		01090-87308
6	B/C	PFS	75	650	2.872	0.3	G1375-87327
6a (direct injection)	B/C	PFS	50	150	0.295		G1375-87300
7	C/-			2000			G1375-87326
8	C/B	PFS	100	200	1.570		G1375-87312
9	B/D	PFS	100	1100	8.639		G1375-87315
10 (Needle)		SST					G1375-87201
11 (Needle seat)							G1375-87101
12	C/D	PFS	25	100	0.049	2	G1375-87320
12	C/D	PFS	50	100	0.196		G1375-87325
13	A/A	SST	170	280	6.355		G1375-87400
14	D/D	PFS	25	220	0.108	4	G1375-87321
15 (Version I, II)	D/C	PFS	25	350	0.172	6	G1375-87322
15 (Version III, IV)	D/C	PFS	25	550	0.270	9	G1375-87323
16 (Version IV)	C/D	PFS	25	100	0.049	2	G1375-87320
16 (Version I, II, III)	C/D	PFS	25	350	0.172	6	G1375-87322
16 (Version I, II, III)	C/D	PFS	25	550	0.270	9	G1375-87323
16 (Version I, II, III)	C/D	PFS	25	700	0.344	12	G1375-87324

*** Fitting A: 5062-2418** **Fitting B: 5063-6593 + 5065-4423** **Fitting C: 5065-4410** **Fitting D: 5065-4422**

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