Agilent Nanoflow LC System for Mass Spectrometry (MS) G2229A

Quick Start Guide

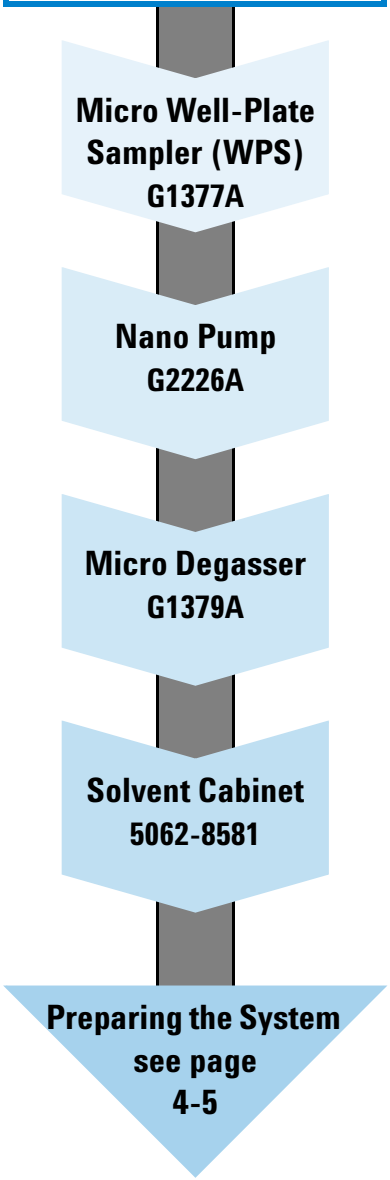
In this guide

Use this guide to help you install your Agilent Nanoflow LC System for MS. This guide also provides valuable tips and hints for operation of the system. Following these hints will ensure a successful run.

If you need to reorder parts please refer to the tables on the rear page.

Agilent Technologies

1. Stacking the System



Installation Step	How to Proceed
Unpack and install the WPS	• Place the micro well-plate sampler on the bench • Remove the ST safety foam • Connect the power cable • Connect the corrugated waste tube to the seat adapter and the solvent waste port from the leak plane
Unpack and install the nanoflow pump	• Place the nanoflow pump on top of the WPS • Connect the CAN cable between the pump and the WPS • Connect the power cable • Connect the waste tube to the EMPV of the pump
Unpack and install the micro degasser	• Place the micro degasser on top of the pump • Connect the power cable • Connect the solvent tube G1322-67300 between the outlet port of the degasser and the solvent selection valve of the pump
Unpack and install the solvent cabinet	• Place the solvent cabinet on top of the degasser • Place the bottles in the solvent cabinet • At the bottle head assembly G1311-60003 replace the glass solvent inlet filter with a SST solvent inlet filter 1018-60025 • Connect the bottle head assembly to the inlet port of the degasser • Connect the tube from the peristaltic flush pump to the solvent bottle in the solvent cabinet

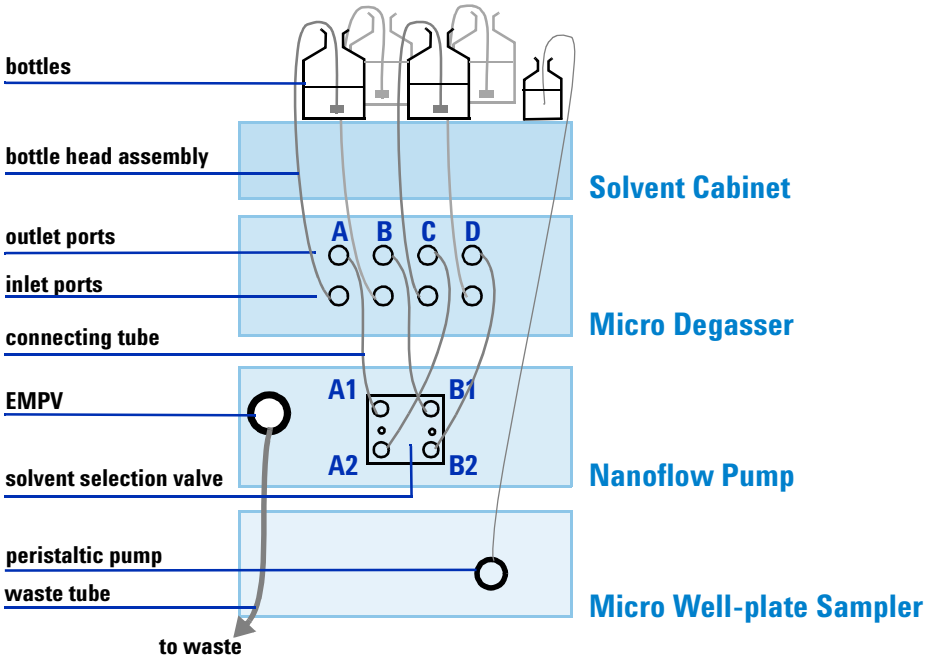


Figure 1 Stacking Overview

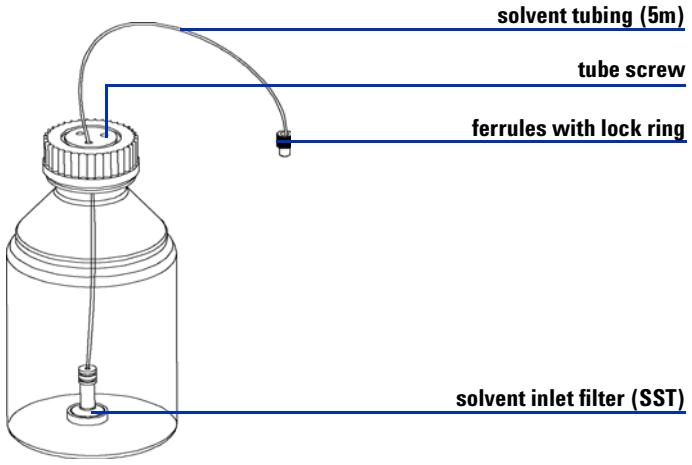


Figure 2 Bottle Head Assembly - Overview

2. Preparing the System

Preparing Solvent

Purging the System

Plumbing the System

Operating tips see page 6

Installation Step	How to Proceed
Prepare solvent	• Prepare 0.1% formic acid in water for channel A • Prepare 0.1% formic acid in ACN for channel B • Prepare 15% methanol, 84% water, 01% formic acid for the washing solvent of the WPS needle
Purge	• Turn ON the micro degasser and the pump • Connect the handheld controller to the pump • Activate the purge mode by selecting: **View>System>Control>Nano Pump>Purge task** at the handheld controller • Set the flow to 2.5 ml/min and purge each channel separately for 4 minutes. • Switch to 50/50 A/B and purge for additional 4 minutes
Connect the capillaries	• Turn ON the micro well-plate sampler • Connect capillary #1 (G1375-87322) (see Figure 3) between flow sensor outlet and switching valve port 1. • Connect capillary #6 (G1375-87323) to switching valve port 6. • Replace the seat capillary # 4 with capillary G1375-87316 provided in the accessory kit • Activate the **Micro** mode by selecting **Setting>Nano Pump > More>Micro Flow** at the handheld controller • Set the flow to 4 µl/min, solvent composition 50/50 A/B. To monitor the flow and pressure at the handheld controller select **Plot>Select>Nano Pump Pressure>More>Nano Pump Flow>More**. Make sure these stabilize at least for 10 minutes before making the next connection. Flow ripple should be less than 15%. Flow and pressure plots should look similar to Figure 4. This procedure can take up to 60 minutes. If after this time the pressure and the flow does not stabilize, there are probably particles at the front end of the capillary. Backflush the tubing to remove them.

Additional Installation Notes

The choice of mobile phase affects chromatography ionization efficiency and sample recovery. Typically mobile phase solvents are water and acetonitrile, both with added organic acid.

- Formic acid causes less ion suppression than TFA
- TFA gives better ion paring / chromatography
- 0.1% formic acid in both water and acetonitrile are good general-purpose mobile phases for peptide analysis.

In the purge mode, the flow goes to waste rather than through the analytical system. You will not damage the system by using the purge mode at 2.5 ml/min.
Purging the system is necessary if:

- It is being used for the first time.
- It was switched OFF overnight or longer.
- The vacuum degasser lines are empty.
- You have changed to a solvent that is immiscible with the previous solvent.

- Before connecting wash both ends with organic solvent and flush before connecting new capillaries to other components
- Avoid air gaps between fittings.
- Do not overtighten, trap (in module doors), or bend capillaries with radius smaller than 4 cm.
- Always install and retighten without flow.
- Use pH lower that 9.

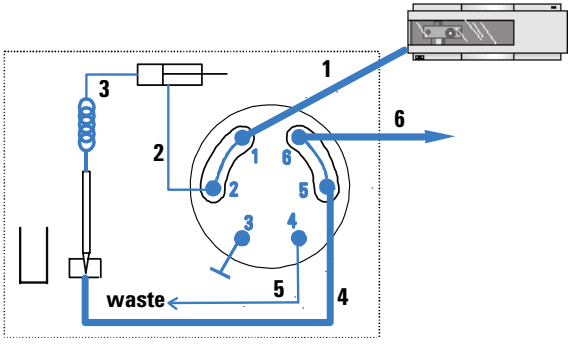


Figure 3 Plumbing diagram (main pass)

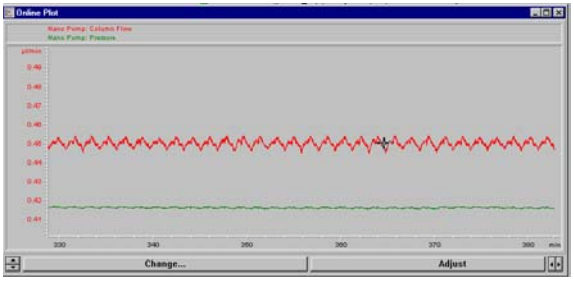


Figure 4 Stability of flow (top) and pressure (bottom)

3. Operation - Tips and Hints

System

- The system pressure of your newly installed system should be 40 - 50 bar under typical conditions (300 nl/min of water with a 50 x 0.075 mm, 3.5 µm column).
- For stable flow, the system pressure must be higher than 20 bar at the pump outlet.
- Check for plugged column capillaries if pressure increases more than 30 %
- For best results, use nanoflow rates from 0.1 µl/min to 1 µl/min.
- In micro mode abnormally high column flow variations are an indication of small particles within the system.
- When using buffer solutions, flush the system with water before switching it off.

Capillaries

- Flush new capillaries before connecting to other components. Wash both ends with organic solvent and be sure the connection is dry before connecting.
- Always install or retighten without flow.
- Do not overtighten, trap (in module doors) or bend with radius smaller than 4 cm.
- Avoid gaps within fittings.
- Use pH lower than 9.
- Replace capillaries if they are bend just after the fitting or anywhere else with a diameter below 4 cm.
- Compare capillary pressure drop to that listed in Table 2. Replace capillary if you have more than 30 % deviation.
- Inspect suspicious capillaries under microscope. Replace those with milky surface.

Vials

The choice of glass versus plastic vials is sample-dependent. If you experience sample recovery problems, you may want to try a different type of vial.
Use the following hints as a guidance:

- Plastic vials are most commonly used.
- Polypropylene inserts and wide mouth vials are recommended.
- Plastic capillary electrophoresis sample vials (300 µl, 9301-0978) can work, but they are opaque and tend to get an air bubble at the bottom of the vial. Air bubbles can cause injection problems.
- Conical polypropylene inserts (100 µl, 5182-05449) are less opaque and less prone to persistent air bubbles at the bottom.

Pump/Degasser

- Use primary flow rate for low solvent consumption.
- After changing solvents, purge each channel for 4 min.
- 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.
- Use clean solvent bottles and solvent.
- Never run without solvent inlet filters.
- Use glass bottled solvents.
- Filter solvents through 0.4 µm filters.
- The default settings (compressibility, flow sensor calibration) are set for water in channel A and acetonitrile in channel B.

Well-plate sampler (WPS)

- The recommended solvent for automatic washing of the autosampler needle is 15% methanol, 84,9% water, 0,1% formic acid.
- Use needle wash.
- Check alignment once a month.
- Ensure comparable pressure drop in a mainpass and bypass once a week.
- Use **bottom sensing** when working with low sample volume.
- For direct injection use **bypass mode.** This leads to a sample transfer time between WPS and column of 3-6 min (300 nl/min).
- Prime flush pump at least once a week for one minute. Check that liquid is draining from the wash port while priming.

For more information on your Agilent Nanoflow System please check the *Nano Pump User Manual* (G2226-90000), the Nano Pump Service Manual (G2226-90100), or the WPS Reference Manual (G1367-90002).

Part Information

Table 1 Fittings and Ferrules

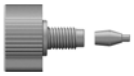
Fitting Type		Name	Description	Conditioning	Part Number
A		Swagelok	1/16″ SST fitting, front and back ferrule	10/pk	5062-2418
B		Lite Touch	4/16″ SST fitting	10/pk	5063-6593
		Lite Touch	1/32″ SST ferrule and lock ring	10/pk	5065-4423
C		Rheodyne	M4 PEEK fitting	6 fitt/2 plug	5065-4410
D		Finger Tight	Double winged nuts and 1/32″ ferrules	10/pk	5065-4422
E		Lite touch detector	M4, 1/16″ SST fitting (male)	10/pk	5063-6593
		Lite touch detector	SST ferrule	10/pk	5063-6592
		Lite touch detector	PEEK sleeve	1/pk	5042-1396

Table 2 Capillaries and Fittings (for item numbers: see Figure 3)

Item	Fitting Type	Material	Diameter (µm)	Length (mm)	Volume (µl)	Pressure-drop for 1µl/min H_2O (bar)	Part number
1	D/C	PFS	25	350	0.172	6	G1375-87322
2		PFS	100	200	1.570		G1375-87312
3	B/D	PFS	100	1100	8.639		G1375-87315
4		PFS	100	150	1.178		G1375-87317
4		PFS	75	150	0.663		G1375-87316
5	C/-			2000			G1375-87326
6	D/C	PFS	25	550	0.270	9	G1375-87323
6	D/C	PFS	25	350	0.172	6	G1375-87322
Restriction Capillary		FS	25	8000	3.927	140	G2226-67300

G2226-90001

Part Number: **G2226-90001**

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