

**Agilent NMR
gHX and 15N -31P [1H]
Nano Probe**

User Guide

Notices

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A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

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1 Introduction

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Nano Probes provide small sample volumes and magic-angle spinning for high-resolution spectra of heterogeneous samples. Nano Probes are also optimized for detection of samples where sample amount is limited.

This manual covers the Nano Probes listed below:

Table 1 Nano Probes

Magnet (MHz)	Bore (mm)	Probe type	Sample size (mm)	Probe p/n (sales order)	Probe p/n (probe label)
400	54	gHX ID	4	95856498	1908995xx
400	54	gHX ID	4	192062300	1908995xx
400	89	gHX ID	4	95856499	1910611xx
500	54	gHX ID	4	95856542	1901926xx
600	54	gHX ID	4	95856638	1901929xx
400	54	^{15}N - ^{31}P {1H}	4	199004059	1911813xx
400	54	^{15}N - ^{31}P {1H}	4	192062400	1911813xx
400	89	^{15}N - ^{31}P {1H}	4	199004058	1921454xx
500	54	^{15}N - ^{31}P {1H}	4	199005075	1911812xx
600	54	^{15}N - ^{31}P {1H}	4	199006101	1921455xx

Proper operation of the Nano Probe requires VnmrJ 2.2C or newer software. If necessary, upgrade console software.



NMR Probes Acceptance Test Procedures Manual

Procedures and information common to most Agilent NMR Probes can be found in the *NMR Probes Acceptance Test Procedures* manual, p/n 91002634.

Refer to the *NMR Probes Acceptance Test Procedures* manual, p/n 91002634, for information on:

- Using the Bayonet Probe Flange
- Connecting the probe
- Mounting the probe
- Connecting the probe to spectrometer
- Recommended starting conditions before NMR testing
- General tuning
- Calibrating the probe
- Testing the probe

Safety Precautions

CAUTION

The dewar sidearm is fragile and can break if the probe is twisted and the VT gas connection puts pressure on the sidearm. When removing the probe with the VT gas connection in place, do not put pressure on the dewar sidearm.

CAUTION

The stator, coil and other elements in the Nano probe can be damaged by contaminants in the gas supplied to the probe. The gas supplied to the probe should be dry, clean, and free of any contaminants whatsoever. If the probe is to be used at higher than room temperature (staying within the specification of the probe, of course) the use of standard air, containing oxygen, could result in oxidation of the coil structure. If the probe is to be used at lower than room temperature (again, staying within the specification of the probe), standard air, which often contains moisture, may allow ice buildup within the probe, which may affect circuit performance and spinning. In addition, often "house air", which is pressurized using a compressor, is notoriously contaminated with oil and metal particles from the compressor. Contaminants such as these cause problems with any NMR probe, especially with the Nano probe. Dry nitrogen is recommended.

CAUTION

Do not force the tuning capacitors to turn past the end of their ranges. The end of the range is indicated by an increase in torque and not necessarily a stop to the rotation. Forced turning can cause serious mechanical damage to the probe, requiring factory repair.

CAUTION

Do not over-tighten the capacitor or inductor stick knob. Over-tightening of the knob could result in damage to the probe.

CAUTION

While the magic-angle stick has been provided with the probe, Agilent recommends that only a qualified Agilent representative perform the magic-angle adjustment. The magic-angle setting is typically a factory-only adjustment. Misalignment of the magic angle will result in a degradation of the probe's observed sensitivity performance.

CAUTION

The VT probe heater lies inline with the bearing gas to the stator. Before shutting off the stator/sample bearing gas valve, turn off the VT heater. Leaving the heater on when the bearing gas is off (for example, while changing samples) can result in heat-related damage to the probe.

CAUTION

Do not exceed spin rates greater than 2500 Hz. Exceeding the recommended spin rate may cause unstable spinning and lead to damage of the sample tube or to the probe.

CAUTION

Place the SETUP/RUN switch in SETUP when changing probes or moving the probe into or out of the magnet. When operating the Nano Probe, set the switch to RUN.



2 Nano Probe Preparation

Changing Samples 10

This section provides information to prepare the Nano probe for NMR experiments.

For information provided in the NMR Probes Acceptance Test Procedures, see [“NMR Probes Acceptance Test Procedures Manual”](#) on page 6.

Changing Samples

Samples can be exchanged using the bayonet probe flange, even with cables and gas lines still attached to the Nano probe.

- 1 Turn off the variable temperature heater.
- 2 Stop the sample spinning using one of the following methods.
 - If under spectrometer control - Set either the airflow or the rotor speed to zero and click **Turn Spinner Off**.
 - Decrease the pressure gradually until sample rotation ceases:
Gradually decrease the Drive gas to 5 psi.
Gradually decrease the Bearing gas to 5 psi.
Gradually decrease the Drive gas to 0 psi.
Gradually decrease the Bearing gas to 0 psi.

Allow about 30 seconds to one minute to ramp the speed down if the sample has been spinning at 2 kHz. Failure to decrease the spinning speed gradually may cause a sample crash and damage to the probe.

- 3 Set the SETUP/RUN switch on the pneumatics router to **SETUP**.
- 4 Detach the pneumatics line at the probe, and remove the probe from the magnet. The tachometer lines may be left attached to the Pneumatics/Tachometer Box if the probe is not to be moved far from the magnet.
- 5 Place the probe in its stand for sample loading and removing.
- 6 Tilt the probe and lightly tap on the shield until the sample falls out into your hand, or carefully pull the sample out with your fingers.
- 7 Inspect the visible part of the stator assembly for any dirt or wear.
- 8 Inspect the new sample
 - a If there is any sign of wear or damage, do not use.
 - b Check that the drive ring mark is present and in good condition.
 - c Wipe off the outside of the sample tube.
- 9 Carefully insert the bottom of the sample into the stator through the shield opening.

The sample should easily drop into place with the convex part of the drive ring stopping against the concave part of the stator.

- 10 Insert the probe into the magnet and make all connections.
- 11 Keep the samples stored in the appropriately marked vials in the probe and rack stand.

CAUTION

The dewar sidearm is fragile and can break if the probe is twisted and the VT gas connection puts pressure on the sidearm. When removing the probe with the VT gas connection in place, do not put pressure on the dewar sidearm.

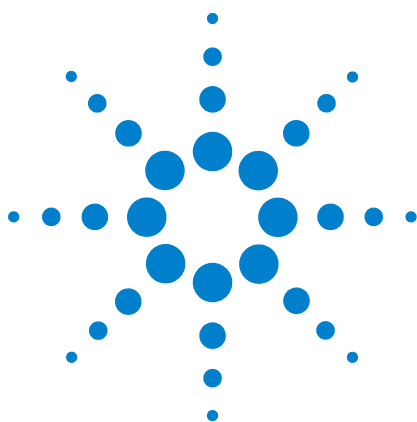
CAUTION

The VT probe heater lies inline with the bearing gas to the stator. Before shutting off the stator/sample bearing gas valve, turn off the VT heater. Leaving the heater on when the bearing gas is off (for example, while changing samples) can result in heat-related damage to the probe.

CAUTION

Place the SETUP/RUN switch in SETUP when changing probes or moving the probe into or out of the magnet. When operating the Nano Probe, set the switch to RUN.

2 Nano Probe Preparation



3 Pneumatics/Tachometer Box Connections

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This section provides information on Pneumatics Router connections to the Nano probe.

Connecting to the Tachometer Module

The Tachometer module is a separate unit mounted on the magnet using the base plate and back plate, see [Figure 2](#).



Figure 2 Mounting the Tachometer module

- 1 Connect the tachometer optical input to the probe. The fiber optic cable requires a "CPMAS board optic cable" adapter to connect it to the Nano Probe. The cable ends must seat correctly to guarantee accurate spin rates.
- 2 Connect the tachometer control port to the pneumatic router tach port using the DB9 cable.
- 3 Turn the 3-position switch to **optical input** (all the way to the right).

Connecting to the Pneumatics Router

This section describes how to connect the Pneumatics Router to the Nanoprobe, see [Figure 3](#).

Connect the air lines

Each line has a number that corresponds to a number on the Pneumatics Router-air connections panel. Connect the air lines by pressing the lines into the quick-connect outlets.

- 1 Connect the pneumatics router-DRIVE AIR port to the probe-DRIVE AIR port. Attach the female quick-disconnect from the air extension to the male quick-disconnect Drive connector on the probe box.
- 2 Attach the female ball-end of the ball joint connector to the male ball-end of the probe dewar. Secure with the pinch clamp. This is for bearing and VT air operation.

If the system has the EZVT option:

- 1 Connect the fitting closest to the silencer on the EZVTTM to LOW TEMPERATURE on the Pneumatic Router.
- 2 Connect the Probe one-touch fitting on the EZVT to HIGH TEMP on the pneumatics router. The VT gases provide bearing gas for the nano sample.

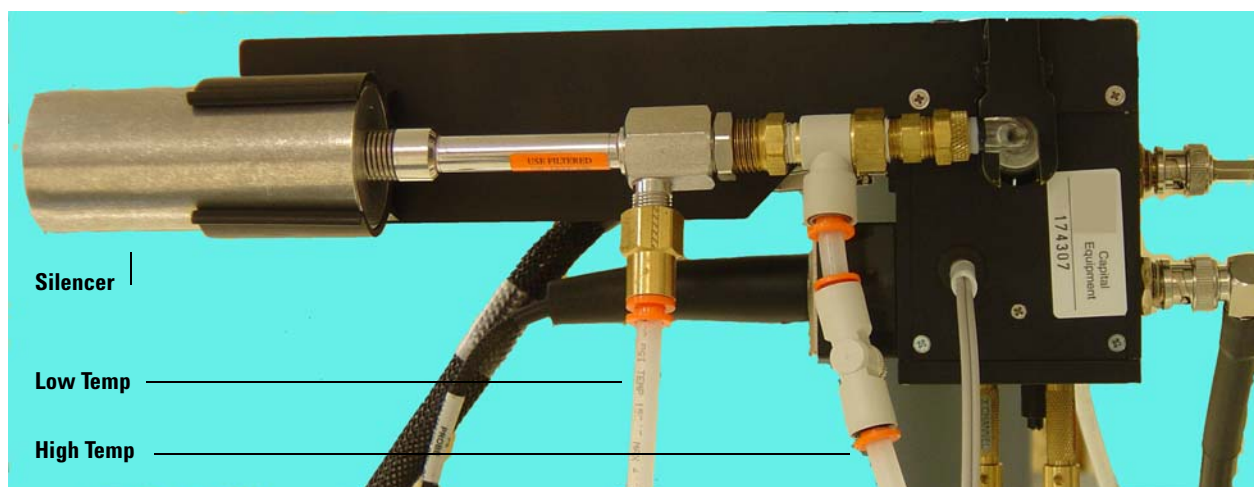


Figure 3 Probe gas connections

CAUTION

The stator, coil and other elements in the Nano probe can be damaged by contaminants in the gas supplied to the probe. The gas supplied to the probe should be dry, clean, and free of any contaminants whatsoever. House compressed air often contains moisture, and is notoriously contaminated with oil and metal particles from the compressor. These contaminants cause problems.

CAUTION

Oxidation of the coil structure could result if the probe is used at temperatures above room temperature with air as the cooling gas. Use dry nitrogen in place of compressed house air.

CAUTION

Ice buildup or condensation within the probe could occur if the probe is used at temperature below room temperature and adversely affect circuit performance and spinning. Use dry nitrogen in place of compressed house air.

CAUTION

Damage to the probe could result if the probe is operated outside of the specified sample temperature range.

CAUTION

Heat-related damage to the probe can result if the heater is left on while the bearing gas is off (for example, while changing samples). Turn off the VT heater before shutting off the stator/sample bearing gas valve. The VT probe heater lies in line with the bearing gas to the stator.

Bearing/Temperature Pressure and Drive Pressure Set Up

CAUTION

Place the SETUP/RUN switch in SETUP when changing probes or moving the probe into or out of the magnet. When operating the Nano Probe, set the switch to RUN.

Bearing/VT Pressure regulator and flow meter set up

- 1 Set the pressure regulator to 30 psi.
- 2 Manually adjust the flow meter to start and stop the rotor. Typical flow rate is between 12-15 lpm.

Drive pressure regulator set up

This is the initial set up, to limit the drive pressure regulator to 30 psi. This should be done prior to spinning the Nano Probe's sample rotor.

- 1 In Open Loop mode, enable **Set spinner air flow instead of speed**.

The slider bar setting is from 0 (closed, no air flow) to 65536 (fully open, maximum flow rate).

- 2 Set the slider to **65536** and adjust the pressure regulator to 30 ±2 psi.

Typical pressure range for bearing/VT and drive is 10-15 psi. The pneumatic router is now set up for Nano Probe operation.

Spinning setup

- 1 Place the Nanoprobe securely on its stand.
- 2 Verify that the probe is attached to the pneumatics router and that the air lines to EZVT are properly attached, see [Figure 3](#).
- 3 Turn on the console power supply.
- 4 Launch VnmrJ on the console PC from the vnmr1 account.
- 5 Set the SETUP/RUN switch on the front of the pneumatic router to **SETUP**.

NOTE

The Pneumatics Router software may need to be reset if the LEDs on the flow meters are flashing. To reset the Pneumatics Router software, click the **eset pneumatic fault** software button.

- 6 Set the Pneumatics Router regulators to the appropriate values, see [Table 2](#). See the figure for locations of meters and regulators:

Table 2 Pneumatics router regulator settings

Regulator	Value
a Purge regulator	40 psi
b Shim purge flow meter	6-8 lpm
c Probe purge flow meter	12-17 lpm (not used with Nano Probes)
d Upper barrel purge flow meter	0 (not used with Nano Probes)
e Anti-vibration regulator	60 - 80 psi
f Temperature pre-regulation regulator	85 psi
g PN temperature regulator	30 - 35 psi
h Upper barrel regulator	40 to 50 psi (not used with Nano Probes)
i Drive Air Manual	Set to fully open for automatic operation.
j Solids gas flow	This sensor indicates the Bearing/VT air for the probe. In normal operation, it is in the 12-18 lpm range. Close it to stop the sample spinning when changing samples.
k Liquids flow meter	Not used with the Nano Probe.

3 Pneumatics/Tachometer Box Connections

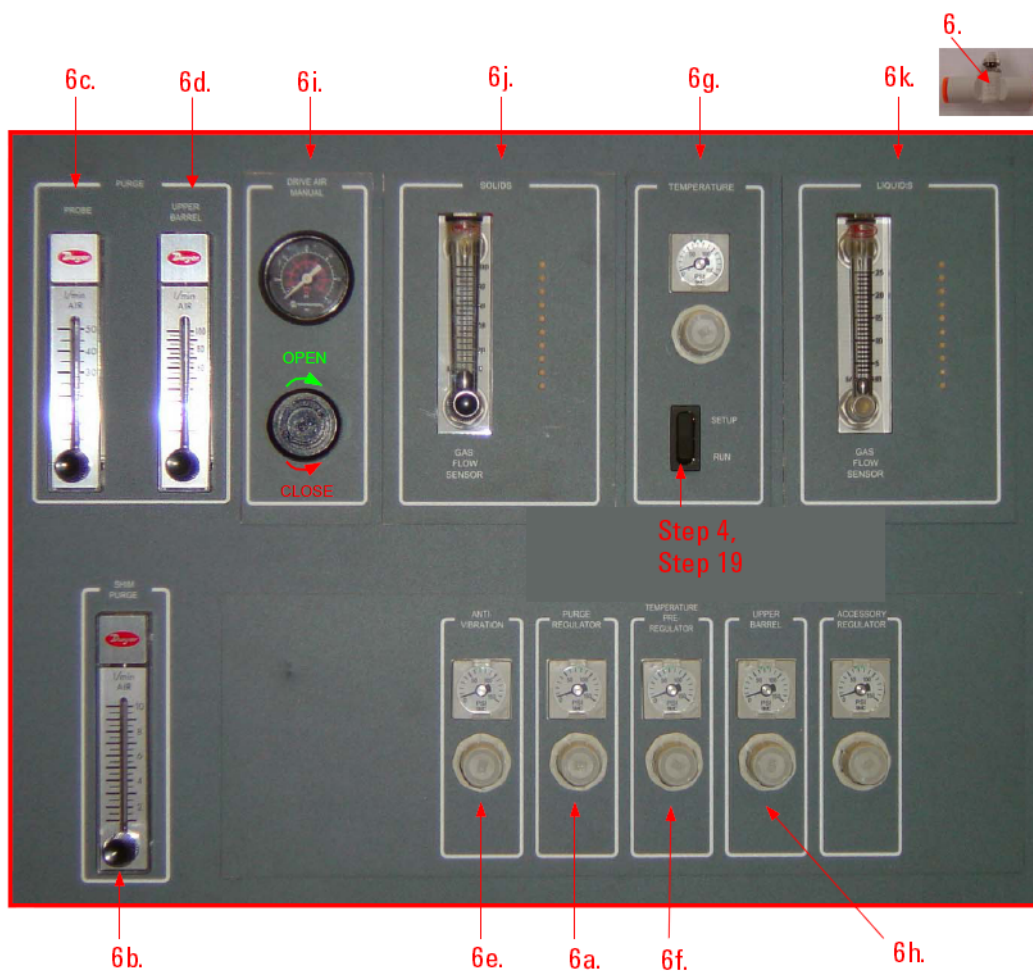


Figure 4 Bearing Air Setup

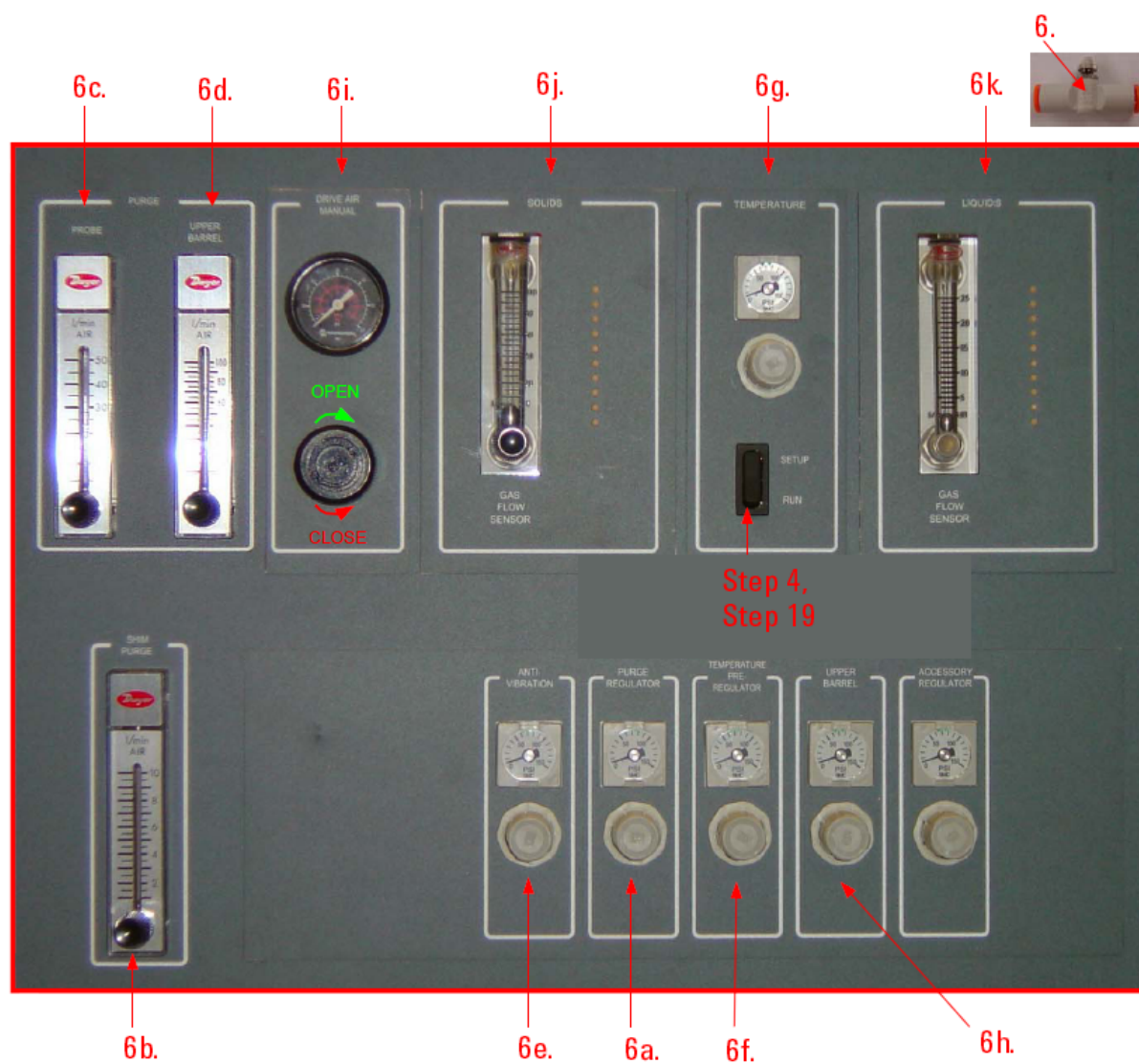


Figure 5 Bearing Air Setup

- 7 Fully open the inline-regulator at the input of the flow meter inside the pneumatics router (min-VT valve), see Figure 6.



Figure 6 Min-VT valve

8 Create a probe file for the Nanoprobe, using the following values:

9 Enter the following values in the probe file fields:

Table 3 Probe file values

Field name value	Text
Probespinmax	2500
Probespintype	tach
Probebearing	0

10 Enter `probe = probe su` on the command line.

11 Prepare a small mouth sample, p/n 190595801 small mouth sample kit.

The sample must withstand -5° C without damage.

12 Insert the sample into the Nano Probe.

13 Set the VT temperature to -5° C in the Spin/Temp window and press the **regulate temp.** button.

Air will flow through the vortex tube.

- 14 EZVT adjustments or settings:
 - a Remove the silencer from the EZVT assembly, see [Figure 3](#).
 - b Use a non-magnetic flat head screwdriver to adjust the proportioning valve at the end of the vortex tube, see [Figure 7](#).



Figure 7 Proportioning valve

- 15 Set the spin rate between 500 and 1000 Hz. The spin rate should stabilize ± 10 Hz.
- 16 Check the following if the sample is spinning and the tachometer **display = 0**:
 - a Verify that the switch on the tachometer is set to optical input.
 - b Flip the optical fibers on the input to the tachometer box.
 - c Adjust the variable resistor on the tachometer until a stable count is shown on the LED display.
- 17 Verify that the spin count in the lower left hand corner of the VnmrJ window is the same as the value displayed on the tachometer module. The low temp VT should read between -8° to -4° C. The settling time may vary due to ambient temperature conditions.
- 18 Record the spin rate with the bearing air set for low temperature VT operation and spinning smoothly here _____.

3 Pneumatics/Tachometer Box Connections

- 19 Click **Temp. Off** in the temperature control panel. Output air from the Pneumatics Router will switch from low to high.

For EZVT:

- a Adjust the inline regulator at the end of the EZVT assembly within 25 Hz of the recorded value in [step 18](#).
 - b Re-attach the silencer to the vortex assembly.
- 20 Set the SETUP/RUN switch on the front of the pneumatic router to **RUN** in preparation for the following tests.

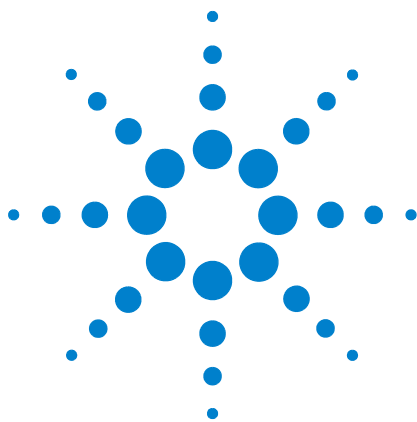
Testing VT and Spin Control

- 1 Set the **temperature** slider in the **Spin/Temp** control panel to 0 °C and select **Regulate Temp**. The temperature should change at a rate of roughly 12 °C per minute. Ensure that the system reaches 0 ± 0.1 °C and is stable for a period of at least 10 min.
Track the changes in temperature by double clicking **Temp** in the corner of the screen to start the chart recorder.
- 2 Set the **temperature** slider in the **Spin/Temp** control panel to 50 °C and press **Regulate Temp**. Ensure that the system reaches 50 ± 0.1 °C and is stable for a period of at least 10 minutes.
- 3 Set the **temperature** slider in the **Spin/Temp** control panel to 30 °C and press **Regulate Temp**. Verify that the system reaches 30 ± 0.1 °C and is stable for a period of at least 10 min.
- 4 Move the slider to 2500 Hz and press **Regulate Speed**.
After a brief delay the system will automatically increase the drive air to ramp up the spin rate to 2500 Hz (± 10 Hz). This usually takes 60 to 80s.
- 5 Double click **Spin** in the bottom left corner of the screen to view the ramping profile.
- 6 Allow approximately 5 minutes for the spin to stabilize.
- 7 Monitor the spin rate to verify that it spins at 2500 Hz (± 10 Hz) for 10 min.
- 8 Set the temperature slider in the **Spin/Temp** control panel to 50 °C and press the **Regulate Temp** button. Allow the temperature to stabilize at 50 °C.
- 9 Press **Spin OFF** and let the system settle back down to the bearing air spin rate.
- 10 Set a new spin rate of 2000 Hz and **regulate spin**.
- 11 Allow approximately 5 min. for the spin to stabilize.
- 12 Monitor the spin rate to verify that it spins at 2000 (± 10 Hz, ± 0.1 °C) for 10 min.
- 13 Press **Spin OFF** and let the system settle back down to the bearing air spin rate.
- 14 Set the **temperature** slider in the **Spin/Temp** control panel to 0 °C and press **Regulate Temp**. Allow the temperature to stabilize at 0 °C.
- 15 Click **regulate spin** to spin the sample back up to 2000 Hz.

- 16 Allow approximately 5 min. for the spin to stabilize.
- 17 Monitor the spin rate to verify that it spins at 2000 (± 10 Hz, ± 0.1 °C) for 10 min.
- 18 Press **Spin OFF** and **Temp OFF** in the **Spin/Temp** control panel.
- 19 Set the SETUP/RUN switch on the pneumatics router to **SETUP** mode.
- 20 Close the solids flow meter and remove the sample from the probe.
- 21 The probe is ready for installation to the magnet and ATP tests.

CAUTION

Place the SETUP/RUN switch in SETUP when changing probes or moving the probe into or out of the magnet. When operating the Nano Probe, set the switch to RUN.



4 Spinning Samples

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These drive ring and sample tubes are designed with 4-place dimensions to help ensure trouble-free spinning, but variations within the tolerances might still occur. Typical spin rates are around 2000 Hz, but certain experiments might require higher or lower spin rates. Spin rates faster than 1000 to 1500 Hz help to get good line narrowing and low-intensity sidebands. Adjust the spin rate to move spinning sidebands out of a particular region of interest or to see if a suspicious peak moves.

For more information on setting up the system for Nano Probe sample spinning, see “[Spinning setup](#)” on page 18.



Manual Spinning

The following is a list of basic steps to spinning samples in the Nano probe. As you become more practiced at spinning samples, you might modify these steps.

- 1 Insert the drive ring and sample tube into the stator. The stator is accessible through an opening in the outer shield.
- 2 With all pneumatic and fiber optic connections in place and the pneumatics/tachometer box regulators at 0 psi, increase the bearing gas supply to about 5 psi.
- 3 The sample should begin to spin slowly. Adjust the rotation trigger, for the fiber optic detection if necessary.

If the Pneumatics/Tachometer Box does not register any speed, but the sample is obviously spinning:

- a Check the paint markings on the drive ring.
 - b Make sure that the fiber optic connectors are properly connected (only handle the fiber optic cable by the black connectors).
 - c If using a Pneumatics / Tachometer Box, try adjusting the potentiometer until the proper speed is registered.
- 4 Slowly increase the drive gas pressure. The sample begins to spin in the low-speed regime (less than 800 Hz). With increased drive gas pressure (2 to 10 psi), a rapid increase in spin speed and a high-pitched tone indicate that the sample is now spinning in the high-speed regime (greater than 1500 Hz). Reduce the drive pressure and then slowly increase the drive pressure again if the sample does not transition from low to high speed. Adjusting the bearing pressure can also help.
 - 5 Increase the bearing gas pressure to 10 psi.
 - 6 Adjust the drive gas pressure to set the desired sample spin rate.

Stopping Spinning

- For spectrometer control, set either the airflow or the rotor speed to zero and click **Turn Spinner Off**.
- Decrease the pressure gradually until sample rotation ceases.
 - Gradually decrease the Drive gas to 5 psi.
 - Gradually decrease the Bearing gas to 5 psi.
 - Gradually decrease the Drive gas to 0 psi.
 - Gradually decrease the Bearing gas to 0 psi.
- Allow about 30 seconds to one minute to ramp the speed down if the sample has been spinning at 2 kHz.

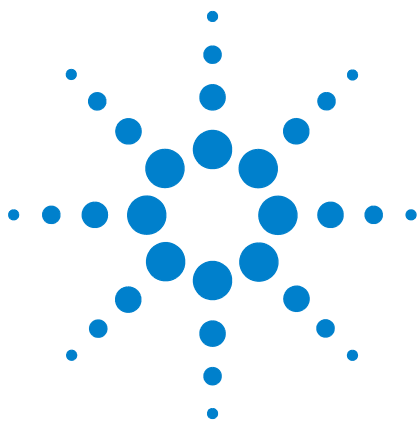
CAUTION

Failure to decrease the spinning speed gradually may cause a sample crash and damage the probe.

Spinning Troubleshooting

Spinning irregularities can be caused by asymmetry in the sample tube, the drive ring, or otherwise damaged sample tubes. Misbalanced samples can also cause spinning problems.

A properly fitting drive ring will not come off while the sample is spinning. If the ring is too loose on the tube, it will probably be out-of-balance when spun. A new drive ring and sample tube combination may improve the situation if repeated attempts at spinning are unsuccessful. Some drive rings seem to spin better than others, and you may want to reuse those rings for most of your experiments.



5 Sample Preparation

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Nano probe sample tubes are precision glass tubes containing a volume of approximately 40 μ l of sample. A drive ring is glued onto the end, and the tube is plugged to prevent the sample from leaking. [Figure 8](#) shows a cross section of the Nano Probe sample tube.

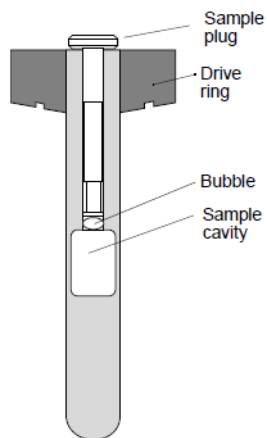


Figure 8 Nano probe sample tube assembly cross section



Choosing a Sample Cell Filling Device

Several devices can be used to fill the Nano Probe sample cell:

- The standard glass Pasteur pipette fits into the Nano Probe sample tube, but the pipette has no graduation, which makes transferring precise amounts difficult.
- Costar plastic pipette tips (10800-492-1110, catalog No. 4853, rounds, elongated, 1 to 200 μL , tip diameter 0.5 mm) fit into a Nano Probe sample tube and are repellent, (non-wetting) with enough solvents to typically transfer the entire sample.
- A standard Hamilton syringe works well - with a metal needle and plunger (10 μL syringe, J&W Scientific Part No. 705-2001) or with a metal needle and gas-tight plunger (10 μL syringe, J&W Scientific Part No. 705-2112).
- Large-capacity Teflon needles work well - 90 μL capacity Teflon needle (Wilmad Part No. 90622; Sigma Part No. Z11735-8, 12-inch, 14-gauge). Large-capacity Teflon needles must be used with a Luer-Lock syringe, preferably a gas-tight model such as a 500- μL Hamilton Luer-Lock Gas-Tight syringe (Wilmad Part No. 81220).

These long needles, especially when equipped with larger-volume syringes, are good for washing out or irrigating Nano Probe sample cells. Resin slurries, even if made in non-polar solvents, can often be fully removed with a jet of tap water delivered with this long-needle, high-volume syringe combination.

Filling with Liquid Samples

- 1 Prepare the liquid sample to be tested and use a pipet to transfer approximately 40 μl into the sample tube.

These sample tubes have small cavities (see [Figure 9](#)) that are easily reached with standard Pasteur pipets. A 40 μl liquid sample should just fill the bubble cavity within the sample tube. The sample plug may have insufficient space to be fully inserted if the sample extends into the constricted region (neck) of the sample tube. A sample plug that is not fully inserted can cause spinning problems.

Sample tubes can have a volume variability of up to 10 μl , so an alternative method of filling the sample tube is to fill the tube cavity with a known quantity of sample (for example, 20 or 30 μl) and fill the remainder of the tube cavity with solvent.

- 2 Insert a sample plug into the sample tube hole, as described in [“Installing Nano Sample Plugs Using the Plug Tool”](#) on page 37. Firmly press in the plug all the way.

Do not bend the sample plug. Bending the sample plug makes insertion difficult. Twist the plug during insertion.

Sample tubes can also be permanently sealed, as described in [Permanently sealing sample tubes, page 37](#).

The sample plugs have two regions of tight fitting diameters to achieve redundant sealing when the plug is inserted all the way. Clean up any excess sample that may have squeezed out.

A bubble decreases the amount of sample in the tube (thus decreasing the signal intensity) but usually has little effect on the NMR otherwise. Eliminating a small bubble from the sample is difficult, but careful sample metering and subsequent spinning in a centrifuge may minimize the bubble.

- 3 Insert the sample tube into the rotor drive ring, with the flat end of the drive ring at the same end as the access hole of the sample tube.
 - a Check the location of the drive ring relative to the sample cavity by inserting the sample tube (with drive ring) into the sample depth gauge, as shown in [Figure 9](#).

The ring is positioned properly when the sample tube cavity is aligned with the hole in the gauge.

- b Adjust the positioning screw in the bottom of the alignment fixture so that the sample cavity is in the window when the sample tube rests against the screw, as shown in [Figure 9](#).

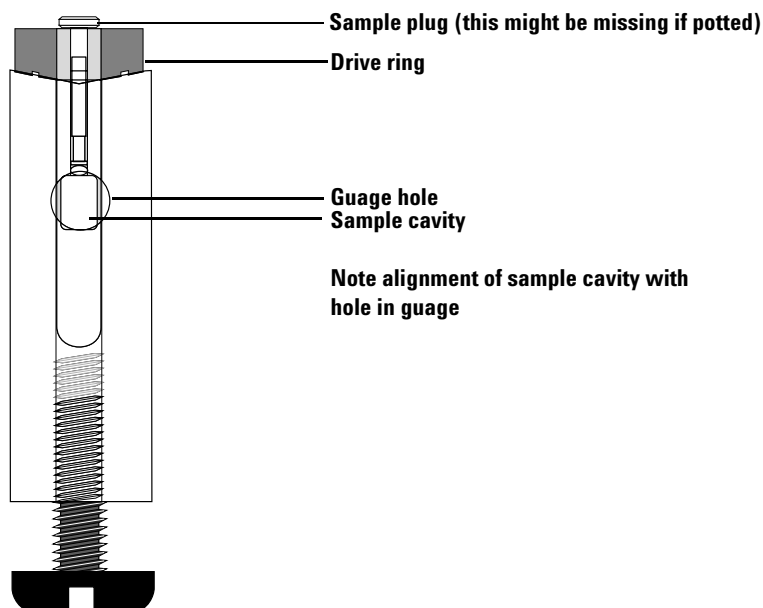


Figure 9 Nanoprobe sample depth gauge with sample tube.

- c Push the drive ring down onto the shaft of the tube until the drive ring seats against the conical surface of the alignment fixture.
Drive rings are reusable. Remove a drive ring using the extraction tool provided.
- 4 Mark new drive rings with a black stripe. The mark should be at least 3/16-in. wide. Avoid paint build-up in the drive flutes on the bottom of the drive ring. Use the ink and paint supplied to touch up a damaged mark. Apply colors in thin layers to avoid un-balancing the sample tube assembly.
Do not use a black magic marker. The optical system does not see the surface marked with a black magic marker as black and it will not function correctly.

- 5 Spin test samples with the probe outside of the magnet before running an experiment:
 - a Place the probe in the probe stand.
 - b Connect the drive gas and tachometer cable as described in “[Connecting to the Tachometer Module](#)” on page 15.
 - c Ramp up the pressure and observe the spin rate as described in “[Manual Spinning](#)” on page 28.
- 6 Remember to wipe off the outside of the sample tube. Fingerprint oils and water are easily detectable with NMR if the sample is dilute and nt is a large number.

CAUTION

Do not exceed spin rates greater than 2500 Hz. Exceeding the recommended spin rate may cause unstable spinning and lead to damage of the sample tube or to the probe.

Filling Semi-Solid Samples

The following steps describe a method for filling nano sample tubes with semi-solids, such as seeds, food products, resin-beads:

- 1 Insert small semi-solids into the sample tube using a Pasteur pipette as a kind of scoop.
- 2 Insert larger, dry semi-solids into the sample tube by dropping them in using a small tweezer.
- 3 ‘Wet’ semi-solids usually can be added to the sample tube using a Pasteur pipette as a scoop and a fine gauge wire to push the contents into the tube. Care should be taken to keep the neck as clean as possible.
- 4 Use either a necked-down Pasteur pipette (having a smaller diameter than the sample tube opening) or a 10 μ L syringe to add the solvent as desired. Some semi-solids can swell when you add solvent, so you might want to fill only about 15% of the tube with sample and the rest with solvent. Underfilling sample tubes is better than overfilling sample tubes. Bubbles are usually of no consequence with the Nanoprobe.
- 5 Seal the sample, see [“Filling with Liquid Samples”](#) on page 33. Sample tubes can also be permanently sealed, as described in [“Permanently sealing sample tubes”](#) on page 37.
- 6 Remember to wipe off the outside of the sample tube. Fingerprint oils and water are easily detectable with NMR if the sample is dilute and the number of acquisitions is a large number.

Installing Nano Sample Plugs Using the Plug Tool

Nano sample plugs may bend when inserted into the sample tubes. To avoid this problem, use the plug tool illustrated in [Figure 10](#). This tool has two usable ends, one thicker than the other. The tool acts like tweezers to grab the plug when finger clamping pressure is applied. It supports the plug while the plug is being pushed into the tube.

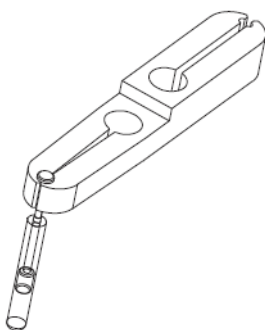


Figure 10 Plug tool for inserting nano plugs

Inserting the sample plug

- 1 Start the plug into the hole in the sample tube.
- 2 Lightly grasp the plug with the tool using the thicker end.
- 3 Put your index finger over the head of the sample plug and push it directly into the sample tube until the bottom of the plug head touches the tool surface.
- 4 After the plug has been inserted as far as possible using the thick end of the tool, remove the tool, flip it around, and grasp the plug with the thin end of the tool.
- 5 With your finger, push the plug straight into the sample tube until the plug head touches the tool surface.
- 6 Remove the tool and push the plug in the rest of the way.

Permanently sealing sample tubes

Sample tubes can be permanently sealed using the following

steps.

- 1 Insert the sample plug so that the first tight region is within the sample tube.
- 2 Trim the sample plug even with the top of the sample tube.
- 3 Continue to insert the severed plug into the sample tube until seated. Remove the top portion of the sample tube.
- 4 Fill the void above the plug with low-viscosity, two-part epoxy (for example, Tra-Con BA2113). Use a syringe and needle or pipette to fill the void from the bottom up to minimize bubbles. Do not overfill. Allow to cure.

Using the Sample Cleaning Holder

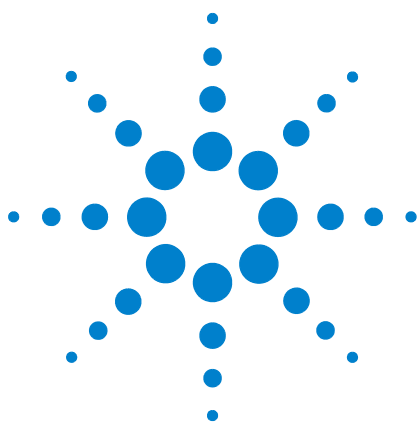
The nano sample cleaning holder enables nano sample tubes to be held upside-down to facilitate cleaning. In use, tubes are held by friction in the holder, which is clamped so that the tubes can be syringed with solvent. The solvent drips into a beaker held underneath.

- 1 Clamp the 1/2-inch rod in a standard laboratory clamp so that the holes in the black plastic part point downward.
- 2 Push the sample tubes to be cleaned into the holes in the black plastic part so that the tube opening faces downward.

The tubes do not need to be fully inserted—just far enough to hold them upside-down.

- 3 Syringe cleaning solvent into the tubes until clean.
- 4 Allow tubes to dry and remove.

5 Sample Preparation



6 Tests

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For information provided in the NMR Probes Acceptance Test Procedures, see [“NMR Probes Acceptance Test Procedures Manual”](#) on page 6.

Tuning Nano Probes

See the *NMR Probes Accentance Test Procedures*, p/n 91002634, for general tuning instructions.

For knob locations, see [Figure 11](#).

Nano probes should be tuned with the sample installed and spinning.

CAUTION

Do not force the tuning capacitors to turn past the end of their ranges. The end of the range is indicated by an increase in torque and not necessarily a stop to the rotation. Forced turning can cause serious mechanical damage to the probe, requiring factory repair.

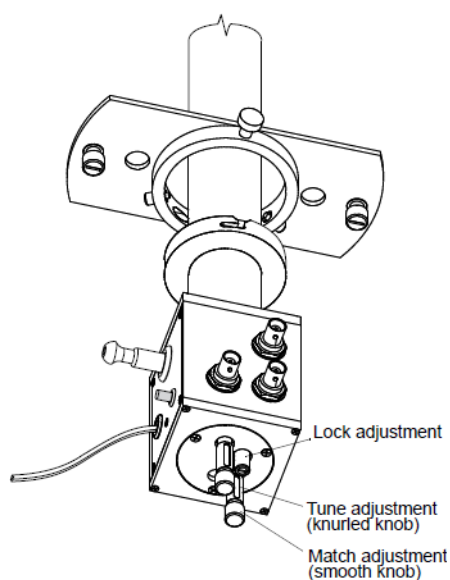


Figure 11 Nano probe tuning controls

Calibrating and Testing Probes

See the *NMR Probes Acceptance Test Procedures*, p/n 91002634, for probe calibration and testing information.

Nano Probes specific instructions

Instructions unique to this probe are as follows:
PFG Profile and Recovery for Nano Probes, use an RF sensitivity window = 0.48 cm

For the applicable acceptance tests, please see the specifications for your probe.

CAUTION

While a magic-angle stick has been provided with your probe, Agilent recommends that only a qualified Agilent representative perform the magic-angle adjustment. The magic-angle setting is typically a factory-only adjustment. Misalignment of the magic angle will result in a degradation of the probe's observed sensitivity performance.

Timing of Pulse Sequence Events

Nano probe data acquired using multipulse experiments will often be improved if the timings of certain pulse-sequence events (i.e. delays and gradient pulses) are set to integral multiples of the rotor period.

Shimming with Nano Probes

Nano probe shimming is very different from typical liquids shimming. Initially, the shimming of this probe may seem difficult, but after a little experience, shimming will seem no more difficult than shimming any other probe. Nano probes are shimmed only when the sample is spinning.

Establishing a basic shim set

- 1 Insert one of the following doped samples:
 - For Proton-observe Nano Probes, use doped D₂O.
 - For X-observe Nano Probes, use doped ASTM.
- 2 Insert the probe into the magnet.
- 3 Select spin rates between 2000 and 2500 Hz.
- 4 Tune the probe and establish lock by adjusting Z0.
- 5 Increase the lock power and lock gain to increase lock signal without saturation.

Saturation can be detected by changing the lock power to a setting 6 dB lower than the previous value and seeing if the lock level halves. If a 6 dB lower setting does not change the lock power downward by about 50%, lock power was initially set too high. In addition, too high a setting will likely result in an appearance of strobing, where the lock level fluctuates rapidly by large amounts. Proton-observe Nano Probes usually end up with lock power settings of less than 10, while X-observe Nano Probes require higher lock power settings.

- 6 Adjust the lock phase to maximize lock level.
Repeat this adjustment after some shimming has occurred.
- 7 Start with a 5 mm probe shim set if there are no pre-existing shim sets for a Nanoprobe. Use a good pre-existing nanoprobe shim set if one exists.
- 8 Use only the transverse (radial) shims (i.e., X, Y, ZX, and ZY) and adjust the shims to maximize lock level.
- 9 Find the 90-degree pulse width value.

Final shimming procedure

- 1 Change samples to your preferred shimming sample.

Proton Nano Probes typically require the chloroform sample, while X-carbon Nano Probes require the undoped carbon sensitivity sample.

- 2 Retune the probe and set the 90° pulse width value.
- 3 Shim the Nano Probe for the best lock signal level:
 - a Adjust four shims in most cases, namely X, Y, ZX, and ZY. One set of two shims will dominate, like X with ZX, or Y with ZY.
 - b Adjust the shims in pairs, X and ZX followed by Y and ZY to obtain the best lock level. Adjusting ZX usually affects the base of the spectral line in a similar fashion to Z4 adjustments in a conventional liquids probe.
 - c If the current shim is not optimized for the best lineshape and resolution:
Completely adjust the low-order XY shim set (X1, Y1, X2, Y2, XY, X2Y2).
Then adjust the third-order XY shim set (X3, Y3, XZ2, YZ2, ZXY) for narrower 0.55% and 0.11% lineshape measurements.
- 4 Readjust the spinning speed to between 2000 Hz and 2500 Hz to attain the highest lock level after the basic shims are set.
- 5 Optimize the sample spinning speed to your particular sample by changing the spin speed to increase the lock level. Adjust the spin speed in increments of 50 Hz. Wait 30 seconds after the spin rate has changed to allow the sample to settle before determining if the change in speed increased or decreased the sample lock level.
- 6 If the current shim is not optimized for the best lineshape and resolution, completely adjust the low-order XY shim set (X1, Y1, X2, Y2, XY, X2Y2). The third-order XY shim set (X3, Y3, XZ2, YZ2, and ZXY) can also be adjusted for narrower 0.55% and 0.11% lineshape measurements.

Strong spinning sidebands (for example, 300 Hz away from the central peak) might indicate bad spinning. Dropping the pressure and then ramping the pressure up again often cure spinning problems, see “Manual Spinning” on page 28.

Probe Test Results

All specifications are subject to change without notice.

Specifications are for probes installed with current production Agilent, Inc. NMR systems. No guarantee is given that probes purchased for use in systems other than currently-shipping will meet current specifications.

Record the agreed specifications and all test results on the data form: Agilent NMR Probe Test Results

Agilent NMR Probe Test Results

Probe Serial Number	Probe Part Number
Probe Type	
Sales Order	Date
Installer Signature	Customer Signature

NOTE

Not all tests listed on this form will be applicable to your probe. See your probe specifications for applicable acceptance tests.

Table 4 90° Pulse width (Direct or Indirect) and RF homogeneity

Nucleus	Specification	Results	RF homogeneity	Notes (tpwr, pwxlvl, sample)
¹ H				
¹⁹ F				
¹³ C	<input type="checkbox"/> direct			
	<input type="checkbox"/> indirect			
³¹ P	<input type="checkbox"/> direct			
	<input type="checkbox"/> indirect			
¹⁵ N	<input type="checkbox"/> direct			
	<input type="checkbox"/> indirect			

Table 5 Resolution and lineshape (50% / 0.55% / 0.11%)

Nucleus	Specification	Results	Notes (Shim set)
¹ H Spin			
¹ H Spinning sidebands			
¹ H Non-spin			
¹³ C Spin			
¹³ C Spinning Sidebands			

6 Tests

Table 6 Sensitivity

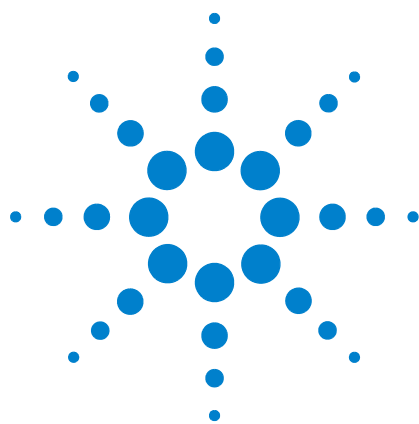
Nucleus	Specification	Results	Notes (sample)
^1H			
^{19}F			
^{13}C			
^{31}P			
^{15}N			

PFG System _____

Table 7 PFG profile and recovery

Axis	Profile (G/cm)	Recovery results	Notes
Z			
X			
Y			

Variable Temperature _____



A Appendix

Setting up Pneumatics for Inova Systems 50

Regulate Spinning Speed from VNMR-INOVA only 51

This section provides information to use Nano Probes on Inova systems.



Setting up Pneumatics for Inova Systems

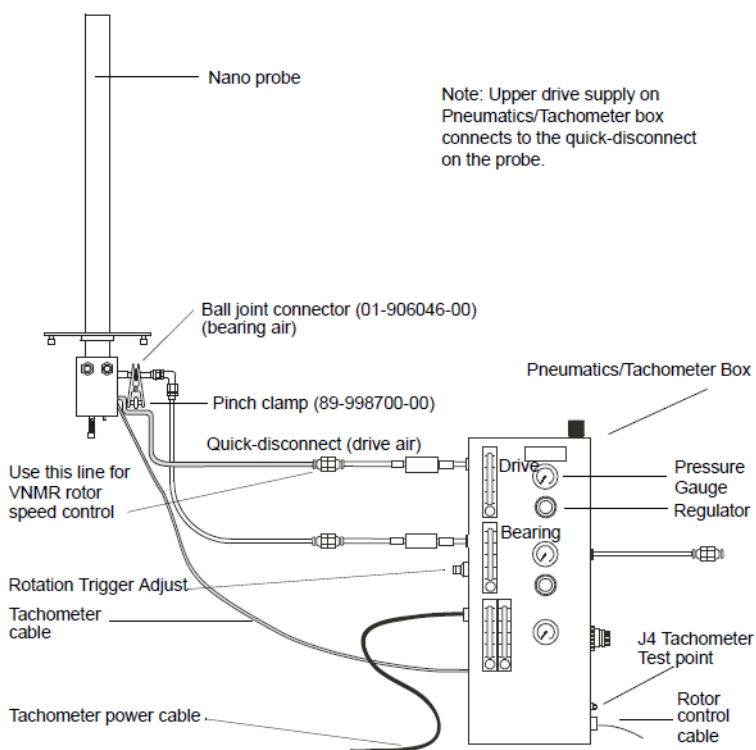


Figure 12 Nano probe pneumatics/tachometer connections - Inova

Regulate Spinning Speed from VNMR–INOVA only

This procedure describes how to regulate spinning speed with VNMR input window. Installation and calibration instructions for the Rotor Speed Controller are provided in the Pneumatics/Tachometer Box, Rotor Synchronization, and Rotor Spin Controller Installation manual.

- 1 Start the rotor speed controller as described in the procedure “[Start the Rotor Speed Controller](#)” on page 52.
- 2 Click on the button next to **Allow spin control in experiment with go**.

This button disables the speed in the Spinner Control window and transfers spin rate control to the spin parameter in VNMR.

- 3 Set up a typical experiment, set the value of spin to the desired speed, and enter **go**.

The spinner regulates at the value of spin. Include a pre-acquisition delay pad to give the spinner time to stabilize. The parameter spin can be included in an array to obtain multiple spin rates in a single experiment. In an array of spin, the pre-acquisition delay is applied before each FID.

- 4 After the experiment is complete, click next to **Allow spin control in experiment with go** to return control to the Spinner Control window.

Using the Rotor Speed Controller

This section provides instructions for using the rotor speed controller. The rotor speed controller can be operated with spinning speed regulation (closed-loop mode) or with a specific airflow setting (open-loop mode).

Alternatively, the air flow can be set to a maximum (65535) and the drive-pressure regulator on the pneumatics/tachometer box can be used for manual control of the spinning speed.

Start the Rotor Speed Controller

- 1 Place a good quality, empty nano sample tube and drive ring in the nano probe.
- 2 Connect the black fiber optic cable to the pneumatics/tachometer box (lower left panel) and set the toggle switch below to V.A. for the nano probe.
- 3 Enter spinner in the VNMR input window to open the Spinner Control window (see [Figure 13](#)).

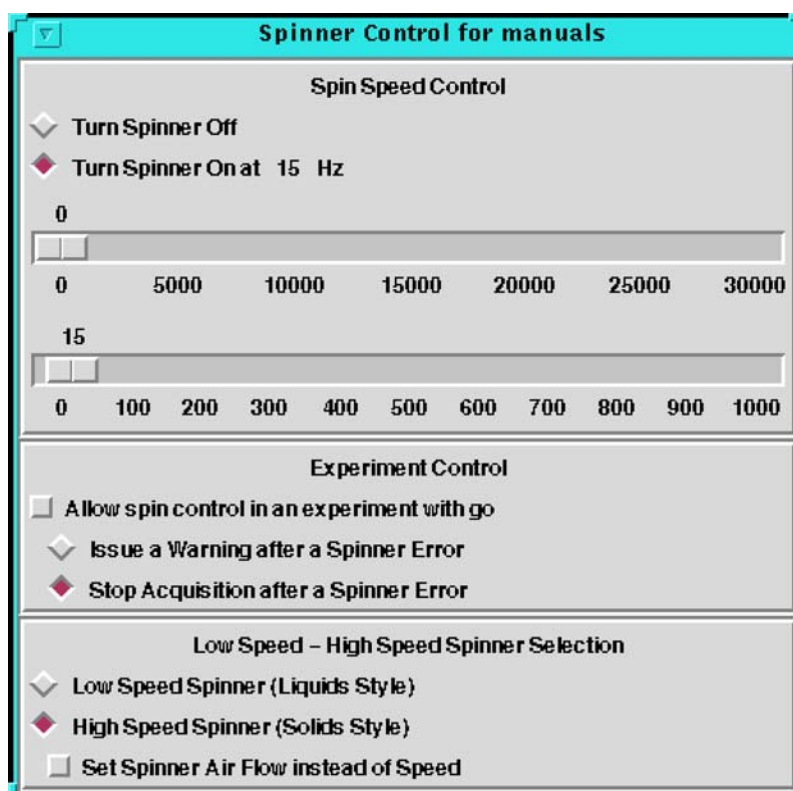


Figure 13 Spinner control window

- a Select the **High Speed Spinner (Solids Style)**.
 - b Select **Set spinner airflow instead of speed**, adjust the two slider bars to zero, and click the Turn Spinner Off button.
 - c The spinner is now in an unregulated or open-loop mode in which the airflow is set directly.
- 4 Increase the drive pressure slowly to a value of 30 psig maximum. Set air flow DAC to 65535 (maximum).

The rotor reaches a maximum speed of about 2500 Hz.

- 5 Once it is engaged, continue to increase the pressure to the maximum value.

Repeat step 3 if the regulator does not engage and recheck the connections to the pneumatics/tachometer box. In any event, do not let a nano sample tube and probe exceed their maximum rating during this process. Once it is engaged, continue to increase the pressure to the maximum value. This must be repeated anytime the drive-pressure regulator has been set to zero.

The rotor speed should be displayed on the Pneumatics/Tachometer box. If not, adjust Rotation Trigger Adjust on the lower left panel (see [Figure 12](#) on page 50) until the speed is displayed. If the speed cannot be observed, check the painting on the drive ring and the fiber optic connections. Also, one can observe the tachometer pulses with an oscilloscope at J4 (see [Figure 12](#) on page 50). The duty cycle should correspond to paint coverage but might vary if the rotor paint has worn thin.

Set the Air Flow

This procedure describes how to set the air flow.

- 1 Set the airflow to 1000 by moving the slider bar to the right. The air flow engages after several seconds.
- 2 Continue to increment the air flow setting in steps until the sample is spinning at about 2500 Hz.

The fine slider bar can be incremented by one when the mouse pointer is clicked in the bar.

The speed displayed in the Acquisition Status window is correct. The speed displayed on the Pneumatics/Tachometer box tends to be slower.

Move the slider bar slowly. Changing the air flow in small steps is good practice if the rotor is spinning. Never set an air flow value that causes a speed that exceeds the rated speed of the nano sample tube and probe. The air flow can also be set before setting the drive pressure. In this case the electro-pneumatic regulator engages at the specified airflow setting.

CAUTION

Do not exceed spin rates greater than 2500 Hz. Exceeding the recommended spin rate may cause unstable spinning and lead to damage of the sample tube or to the probe.

Regulate Spinning Speed

If the rotor checks out, return the airflow setting to zero, select **Turn Spinner Off** and click to disengage the button **Set Spinner Airflow Instead of Speed**. The spinner is now in regulation or closed-loop mode. Enter a spinner speed of 2500 on the slider bars. The spinner should regulate to ± 2 Hz or better within about 30 seconds to 1 minute. Experiment with a number of set points within the rated speeds of the nano sample tube and probe.

In closed-loop mode, the speed can be increased or decreased by any value. The speed is safely ramped to the new value. However, making small changes in speed (1000 to 2000 Hz) is good practice until you are comfortable with the operation and reliability of a particular rotor and endcap combination. Never exceed the rated speed of the nano sample tube and probe.

CAUTION

Do not exceed spin rates greater than 2500 Hz. Exceeding the recommended spin rate may cause unstable spinning and lead to damage of the sample tube or to the probe.



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