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Triple-Resonance NMR Probes

Installation, Testing, and Specifications

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Overview

Triple-resonance probes are specifically designed for demanding applications in biomedical NMR such as heteronuclear 2D, 3D, and 4D triple-resonance experiments on isotopically enriched proteins.

This manual covers the following triple-resonance probes:

Magnet (MHz)	*Bore (mm)*	*Sample Size (mm)*	*Range*	*Probe Part No.*
400 PFG	54	5	$^{1}H\{^{13}C/^{15}N\}$	00-993514-01
400 PFG	89	5	$^{1}H\{^{13}C/^{15}N\}$	01-901580-01
500 PFG	54	5	$^{1}H\{^{13}C/^{15}N\}$	00-992430-05
500 PFG	54	8	$^{1}H\{^{13}C/^{15}N\}$	01-903028-00
500 XYZ PFG	54	5	$^{1}H\{^{13}C/^{15}N\}$	01-901589-01
600 PFG	54	5	$^{1}H\{^{13}C/^{15}N\}$	00-992910-04
600 PFG	54	8	$^{1}H\{^{13}C/^{15}N\}$	01-902809-00
600 XYZ PFG	54	5	$^{1}H\{^{13}C/^{15}N\}$	01-901592-01

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The PFG (pulsed field gradient) versions of the triple-resonance probe adds rapid recovery gradients, up to 65 G/cm along the Z axis, to the probe features. The PFG probe requires the Pulsed Field Gradient Module (Part Nos. 00-992915-00, 00-992916-00).

The XYZ PFG versions of the triple-resonance probe adds X (25 G/cm), Y (25 G/cm), and Z (65 G/cm) gradient capability to the probe features. The XYZ PFG probe requires the XYZ PFG module for UNITY*INOVA* (01-903977-00) or UNITY*plus* (01-903977-01).

To achieve the required level of performance for these specialized experiments, the probe has been optimized in the following ways. Keep these points in mind during use.

- *High-power handling* – The probe can typically handle full pulse power (200 to 300 W) for up to a 180 pulse.
- *Simultaneous pulse conditions* – Find the maximum power (P_{max}) that the carbon channel can take without arcing. Then, for simultaneous carbon and nitrogen pulses, apply the following derating relation:

  $$P_{13_C} + P_{15_N} + 2\sqrt{P_{15_N} P_{13_C}} \leq P_{max}$$

  This derating ensures trouble-free probe operation. However, provided that the *pulses are less than* 100 s *long*, slightly more power (to near the point of arcing) can be applied without damaging the probe.
- *Spin locking conditions* –

Channel	*Gamma B_x (kHz)*	*Max. Pulse Duration (ms)*	*Approx. Power (W)*
^{13}C	7	30	30
^{15}N	2	30	30
^{31}P	2	30	30

- *Decoupling conditions* –

Channel	*Gamma B_x (kHz)*	*Max. Pulse Duration (ms)*	*Approx. Power (W)*
^{13}C	5	150	15
^{15}N	1	150	7.5
^{31}P	1	150	1.5

- *Nonspinning operation* – The probe is intended for cancellation experiments that are typically performed nonspinning. Therefore, nonspinning performance has been optimized relative to spinning performance.
- *Tuning* – Proper tuning is vital to short pulse width performance. *Always tune the probe with no filter in line*. Once tuned, attach the required filter using the shortest cables available.
- *5-mm tuning range* – The 5-mm probe tunes to the specified nucleus on each channel with as much as 0.25 M sample loading. None of the four channels is broadband tunable.
- *8-mm tuning range and sample geometry* – To ensure the highest proton sensitivity at lower salt concentrations, the matching range of the 8-mm probe is limited to 250 mM of salt. Using smaller diameter sample tubes (e.g., 6-mm or 7-mm) can help optimize sample geometry and salt tolerance. For ionic samples in an 8-mm tube, the `pw90` value might increase by as much as 1.5 times for a 100 mM solution and 1.8 times for

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a 250 mM solution. For concentrations higher than 250 mM, using smaller diameter sample tubes or using 5-mm probes might be more efficient. Restricting the sample height by using Shigemi sample tubes also reduces sample loading and thus the degradation of pulse width. The optimum sample height for the 8-mm Triple-Resonance PFG probe is 50 mm.

- *8-mm air flow* – For the 8-mm probe, we recommend using 10 to 15 lpm if VT air flow and 5 to 10 lpm of body cooling air. For longer-duration decoupling, you can increase the body cooling air.

Safety Precautions

CAUTION: **To prevent damage to the probe, be extremely careful when applying spin-locks or high-power decoupling to the probe.**

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

Installing the Probe

1. Make sure the upper barrel and probe mounting flange are installed and adjusted for the probe.
   - The upper barrel should be seated against the room-temperature shim coil.
   - The probe flange set screws should be backed off from the probe shield, allow the flange to slide (with some effort) along the shield.

   Probes used with widebore (89-mm) magnets should be fitted with the widebore adaptor (a white plastic ring shipped in the probe case). Position the adaptor at the appropriate midpoint of the probe shield and secure in place with the three set screws. Placing the adaptor too close to the NMR coil region causes lineshape difficulties.
2. Insert the probe into the bottom of the magnet dewar.
3. Secure the probe flange to the magnet with the thumbscrews.
4. Push the probe up until contact is made with the upper barrel.
5. Apply pressure while rotating the probe until the probe shield key engages; this aligns the PFG pins with the sockets in the upper barrel.
6. Continue to push the probe up until the pins engage and the upper barrel rises slightly (this ensures a good seal between the upper barrel and probe).
7. Press the upper barrel down until it seats into the room-temperature shim coil.
8. Tighten the three set screws in the probe flange against the probe shield. Overtightening the set screws will dent the probe shield.

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Connecting the Probe

To produce simultaneous high-power ^{13}C and ^{15}N pulses (or decoupling) at the probe, the spectrometer must be equipped with a third channel accessory. The third channel, because of the absence of routing relays, typically delivers higher power than the main decoupler and should be connected to the NITROGEN port of the probe.

1. In the lock channel line, install the appropriate ^{2}H bandpass filter listed in the following table. Expect the lock phase to change when the filter is added. Note that the ^{2}H bandpass filter can be left in the system at all times; however, it causes about 20% loss in lock sensitivity.

Magnet (MHz)	*^{2}H Bandpass Filter*
400	BE61-10-8BB
500	BE77-3.8-8BB
600	BE95-12-8BB

   Filter model numbers describe the function of the filter. For example, for model BE77-3.8-8BB, the 77 indicates 77 MHz and “B” indicates bandpass; an “L” instead would indicate low-pass.

2. Connect the low-band decoupler cable according to the appropriate procedure below. Choose the appropriate bandpass filters from the following table:

Magnet (MHz)	*^{13}C Bandpass Filter*	*^{15}N Bandpass filter*
400	BE109-22-8BB	BE40-14-9BB
500	BE135-35-8BB	BE53-15-8BB
600	BE154-40-8BB	BE61-10-8BB

   - *400-MHz systems* – Connect a cable from the B Band port, or the Probe NORMAL port on the magnet leg, to the appropriate bandpass filter. Then attach a cable from the filter to the probe port labeled CARBON.
   - *500-MHz systems with $^{1}H/^{19}F$ OBS preamplifier* – Connect a cable from the Decplr or NORMAL port to the appropriate bandpass filter. Then attach a cable from the filter to the probe port labeled CARBON.
   - *500-MHz systems with dual preamplifier* – Connect a cable from the B Band CM port to the appropriate bandpass filter. Then connect a cable from the filter to the probe port labeled CARBON.
   - *600-MHz systems* – Connect the appropriate bandpass filter to the cable labeled “Indirect Detection” or “L Band Decpl.” Then connect a cable from the filter to the probe port labeled CARBON

3. Attach the third channel cable to a ^{15}N bandpass filter (see the table above for filter model number) and then to the probe connector marked NITROGEN.
4. Connect the ^{1}H channel according to the appropriate step below:
   - *400-MHz systems* – Connect a ^{1}H high-pass filter to the H Band port or 1H/OBD/DEC port. Then connect a cable from the filter to the PROTON port on the probe.

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- *500-MHz systems with $^{1}H/^{19}F$ OBS preamplifier* – Connect a ^{1}H high-pass filter to the PROBE port (J5301) on the preamplifier. Then connect the a cable from the filter to the PROTON port on the probe.
- *500- and 600-MHz probes with dual preamplifier* – Connect a ^{1}H high-pass filter to the H BAND CM or the PROBE port (J5301) on the preamplifier. Then connect a cable from the filter to the PROTON port on the probe.

5. Connect the cable from the variable temperature controller (VTC) to the 9-pin connector (VT Control) on the probe.
6. Connect the VT nitrogen gas hose to the stub tee on the glass dewar projecting to the side at the base of the probe.
7. Connect the body-cooling air hose to the hose barb protruding from the bottom of the probe.

Tuning the Probe

Triple-resonance probes perform best when the sample to be analyzed is placed in the probe during the tuning operation and tuning procedures are repeated every time you change samples.Because the probe channels are slightly interactive, repeat the ^{1}H, ^{13}C, and ^{15}N tuning procedures, checking to see if each channel shows zero reflected power on the tune meter. Make minor adjustments to the probe as necessary.

Tuning the ^{1}H Channel

Alternately adjust the match and tune knobs to find the minimum reflected power on the magnet leg tune meter. The knurled knob with the red PROTON inset label turns the tuning capacitors and the smooth lower knob turns the match capacitor (see Figure 1).

Turning the knurled knob clockwise (as viewed from the bottom of the probe) decreases tune capacitance, while turning the lower (smooth) knob clockwise increases the match capacitance.

Tuning the ^{13}C Channel

Alternately adjust the match and tune knobs to find the minimum reflected power on the magnet leg tune meter. The knurled knob with the white CARBON inset label turns the tuning capacitors and the smooth lower knob turns the match capacitor (see Figure 1). Turning the knurled knob clockwise (as viewed from the bottom of the probe) decreases tune capacitance, while turning the lower (smooth) knob clockwise increases the match capacitance.

Tuning the ^{15}N Channel

Alternately adjust the match and tune knobs to find the minimum reflected power on the magnet leg tune meter. The knurled knob with the yellow NITROGEN inset label turns the tuning capacitors and the smooth lower knob turns the match capacitor (see Figure 1). Turning the knurled knob clockwise (as viewed from the bottom of the probe) increases tune capacitance, while turning the lower (smooth) knob clockwise increases the match capacitance.

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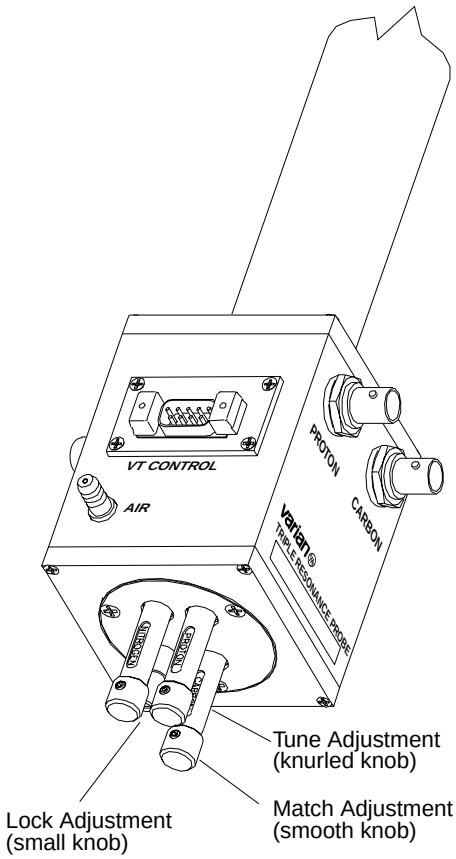


Figure 1. Triple-Resonance Probe Tuning Knobs

Testing Triple-Resonance Probes

This section contains test and calibration procedures for the triple-resonance probes.

- Table 1 lists the tests appropriate for each probe type.
- Table 2 lists the test samples.
- Specifications for Triple-Resonance Probes, page 18, lists test specifications.

^{1}H Observe PW90 and RF Homogeneity

Use this procedure to determine the ^{1}H observe 90° pulse width and rf homogeneity.

1. On 400-MHz systems, make sure the ^{1}H quarter-wavelength cable is installed.
2. Tune the probe using a doped 2-Hz D_2O sample listed in Table 2.
3. Enter **`rtp('/vnmr/tests/shmd2o')`** to retrieve the test parameter set to the current experiment. Enter **`at=2 sw=2000 d1=2 nt=1 lb='n' spin='n'`**.
4. Acquire a normal spectrum and shim the water signal to less than or equal to 3-Hz linewidth at 50%.

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Table 1. UNITY*INOVA* Probe Test Order and Indirect Detection Probe Types

Tests	*400, 500, 600 PFG* $^{1}H\{^{13}C/^{15}N\}$	*500, 600 PFG XYZ* $^{1}H\{^{13}C/^{15}N\}$
^{1}H Observe PW90 and RF Homogeneity, this page	✔	✔
^{1}H Spinning Resolution & Lineshape, page 8	✔	✔
^{1}H Spinning Sidebands, page 9	✔	✔
^{1}H Nonspin Resolution & Lineshape, page 9	✔	✔
PFG Recovery and Profile, page 10	✔ (Z axis only)	✔ (Z, X, and Y axes)
^{1}H Sensitivity, page 13	✔	✔
^{13}C pwx90 Pulse Width and RF Homogeneity, page 14	✔	✔
^{15}N pwx90 Pulse Width and RF Homogeneity, page 15	✔	✔

Table 2. Triple-Resonance Probe Acceptance Test Samples

NMR Test	*Sample*	*Part No.*
^{1}H PW90, ^{1}H sensitivity (5-mm)	0.1% ethylbenzene, 0.01% TMS 99.89% deuterochloroform ($CDCl_3$)	00-968120-70
^{1}H PW90, ^{1}H sensitivity (8-mm)	0.1% ethylbenzene, 0.01% TMS 99.89% deuterochloroform ($CDCl_3$)	00-903508-70
^{13}C PWX90 (5-mm)	1% iodomethane–^{13}C, 1% trimethylphosphite, 0.2% Cr(acac) in $CDCl_3$	00-968120-96
^{13}C PWX90 (8-mm)	1% iodomethane–^{13}C, 1% trimethylphosphite, 0.2% Cr(acac) in $CDCl_3$	00-903508-96
^{15}N PWX90 (5-mm)	2% benzamide-^{15}N, 0.2% Cr(acac) in DMSO–d_6	00-968120-97
^{15}N PWX90 (8-mm)	2% benzamide-^{15}N, 0.2% Cr(acac) in DMSO–d_6	00-903508-97
^{1}H spinning res. & lineshape, ^{1}H spinning sidebands, ^{1}H nonspin res. & lineshape (5-mm)	1% chloroform in acetone–d_6	00-968120-89
^{1}H spinning res. & lineshape, ^{1}H spinning sidebands (8-mm)	1% chloroform in acetone–d_6	00-903508-89
^{13}C sensitivity (5-mm)	40% p-dioxane in benzene–d_6 (ASTM)	00-968120-69
PFG	2-Hz 1% H_2O, 99% D_2O	01-901855-01

5. The water signal should be on-resonance. Place the cursor on the water signal and enter **`nl movetof`** to move the transmitter offset on the water peak.

   The transmitter must be on resonance for the test to be accurate.

6. Determine the correct `tpwr` as follows:

   a. Enter **`tpwr=50 pw=7 ga`**.

   b. After the acquisition is complete, enter **`aph`** to phase the spectrum.

   c. Set the **`pw`** to twice the pw90 specification for the probe and array **`tpwr`** from 48 to 58.

   Observe the spectra as they are being collected. When the peak of interest passes through the null and goes negative, the acquisition can be halted by

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entering `aa`. The `tpwr` value corresponding to the first negative spectrum should then be used for pw90 calibrations.

7. Expand the spectrum so that the quartet is centered on the screen with **`wp=250`**. Enter **`ai`**. Set **`vs`** so that the signal occupies about half the vertical size of the screen. Enter **`vp=50`**.
8. Setup the following array to determine the pw90 for a probe:
   a. Enter **`r1=`*`xx`*`/5`**, where *xx* is the specified pw90 specification for the probe.
   b. Enter **`array('pw',20,r1,r1)`** to create a pw array covering approximately 360 degrees of nutation.
9. Set up the following array to determine rf homogeneity:
   a. Enter **`r1=`*`xx`*`/10`**, where *xx* is the specified pw90 specification for the probe.
   b. Enter **`array('pw',100,r1,r1)`** to create a pw array covering approximately 900 degrees of nutation.
10. Enter **`ga`** to start the acquisition. After the last spectrum finishes, enter **`ds(1)`** to display the first spectrum. Enter **`aph0`** to phase the spectrum, then enter **`ai dssh`** to display the arrayed spectra.
11. Enter **`ds(10)`** (or whatever the spectrum number corresponds to the first maximum spectrum) **`full`** to display only the single spectrum. Determine the amplitude by entering **`peak`** to display the height of the peak (in mm) and the frequency of the peak (in Hz). Record the peak height (in mm) corresponding to the 90° pulse width spectrum.

    Repeat the steps for the spectra numbers corresponding to the 450° pulse width and the 810° pulse width spectra, respectively. Determine the peak amplitudes for the 450° pulse width and the 810° pulse width spectra. Record the peak heights (in mm) corresponding to the 450° pulse width and the 810° pulse width spectra.
12. Calculate the amplitude ratios as a percentage (e.g., (450°/90° ratio)(100) is the rf homogeneity specification) for 450°/90° and 810°/90°. Plot the arrayed spectra. Write down the probe type, probe serial number, and the 450°/90° and 810°/90° percentages on the forms in Specifications for Triple-Resonance Probes, page 18.

^{1}H Spinning Resolution & Lineshape

This test measures the 50%, 0.55%, and 0.11% of the $CHCl_3$ sample. This test and the ^{1}H spinning sidebands test (the next test) *must be passed simultaneously* and both tests plotted together.

1. Put the appropriate $CHCl_3$ sample in the probe and spin it.
2. Enter **`rtp('/vnmr/tests/h1lshp')`**. Enter **`su`** to set up the system hardware.
3. Change **`pw`** to the ^{1}H 90° pulse width (determined from the ^{1}H rf homogeneity section). Change **`tpwr`** to the `tpwr` level to give the ^{1}H 90° pulse width.
4. Enter **`ga`** to acquire the spectrum. Phase the spectrum, then plot it using **`wp=250`**. Enter **`vs=10k`** and plot the expanded spectrum. Make sure the spectrum is normalized.

   If floor vibration results in excessive noise around the base of the peak, **`nt`** can be changed to a larger value (e.g., `nt=4` or `nt=16`); however, if extreme vibrations are present, it may be impossible to measure the lineshape accurately.
5. Measure lineshape as the linewidth of the $CHCl_3$ peak at 50%, 0.55%, and 0.11% of the main peak amplitude. Refer to Figure 2 for the measurement points.

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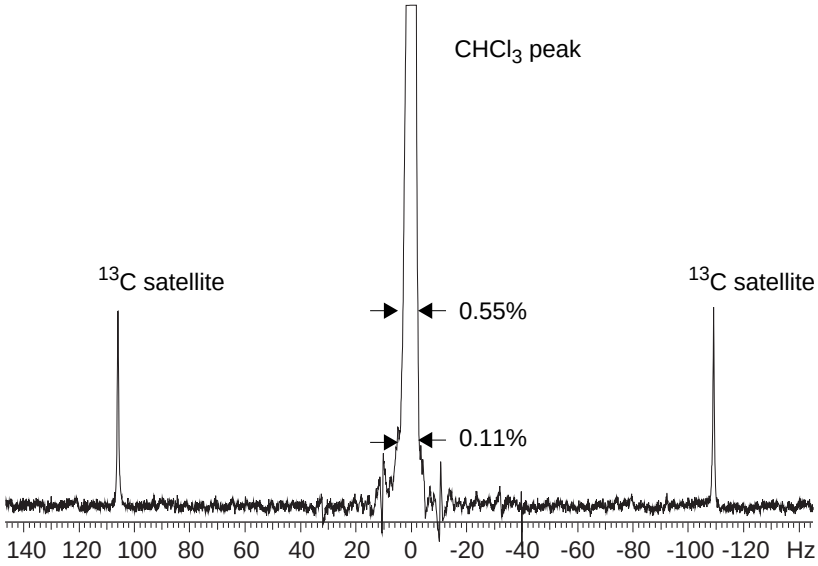


Figure 2. ^{1}H Lineshape Spinning Measurement

6. Write the results for each probe on the forms in Specifications for Triple-Resonance Probes, page 18.

^{1}H Spinning Sidebands

This test and the ^{1}H spinning resolution & lineshape test (the previous test) *must be passed simultaneously* and both tests plotted together.

1. Using the appropriate $CHCl_3$ sample, measure the ^{1}H spinning sidebands on the same spectrum as ^{1}H lineshape.
2. Use the spectra and parameter set from the ^{1}H lineshape test. Plot the spectrum again using a large enough value of `wp` to show all the spinning sidebands.
3. Measure spinning sideband amplitudes as a percentage of the main peak.

   Spinning sidebands occur at frequency intervals on either side of the central peak equal to the spinning rate. The sidebands may not be split.

   The standard test requires `nt=1`. If sidebands meet specifications at `nt=1`, repeating the test at `nt=4` is unnecessary.
4. Write the results for each probe on the forms in Specifications for Triple-Resonance Probes, page 18.

^{1}H Nonspin Resolution & Lineshape

This test measures the 50%, 0.55%, and 0.11% of the of $CHCl_3$ sample.

1. Use the appropriate $CHCl_3$ sample
2. Enter **`rtp('/vnmr/tests/h1lshp')`**. Enter **`su`** to set up the system hardware.
3. Change **`pw`** to the ^{1}H 90° pulse width (determined from the ^{1}H rf homogeneity section). Change **`tpwr`** to the `tpwr` level to give the ^{1}H 90° pulse width.
4. Turn the spinner off and set the spin rate to 0.

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5. Enter **ga** to acquire the spectrum. Phase the spectrum, then plot it using **wp=250**. Enter **vs=10k** and plot the expanded spectrum.

   If floor vibration results in excessive noise around the base of the peak, **nt** can be changed to a larger value (e.g., `nt=4` or `nt=16`); however, if extreme vibrations are present, it may be impossible to measure the lineshape accurately.

6. Measure lineshape as the linewidth of the $CHCl_3$ peak at 50%, 0.55%, and 0.11% of the main peak amplitude. Refer to Figure 2 for the measurement points.

7. Write the results for each probe on the forms in Specifications for Triple-Resonance Probes, page 18.

PFG Recovery and Profile

Z Axis PFG Profile for Performa I,II, and XYZ

It is useful to translate the gradient control in DAC units to G/cm by a constant that represents G/cm-DAC units. The constant is `gcal`, a user, global, real-valued parameter.

1. Use **mp** to move the doped D_2O parameters used in the rf homogeneity test to a new experiment. Join (**jexp**) the experiment.
2. Enter **profile**.
3. Enter the appropriate value for `gzlvl1`:
   - Performa I: **gzlvl1=250**
   - Performa II/XYZ: **gzlvl1=4000**
4. On *Performa XYZ systems*, create the `gradaxis` parameter if it does not already exist:

   ```
   create('gradaxis','string')
   gradaxis='z' su
   ```

5. Enter **ga**. When acquisition finishes, enter **f full dc vsadj**.

   You should see a very wide peak (about 16 kHz for Performa I or about 50 kHz for Performa II/XYZ), similar to Figure 3.

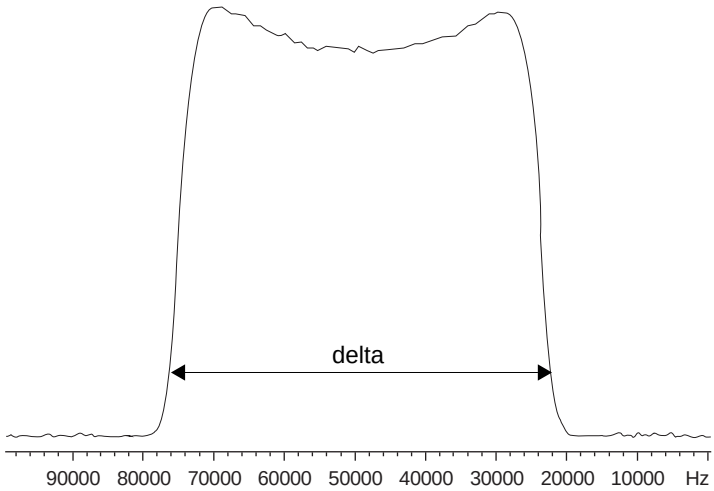


Figure 3. Z-Gradient Profile Measurement Using 1% Doped H_2O

6. Use the cursors to find the width of this peak at 20% of the height.

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7. Enter **setgcal** to convert the width of this plateau (delta) and the gradient strength (gzlvl1) to a gradient strength per DAC value (gcal).

   The setgcal macro needs the physical extent of the rf sensitivity region (1.6 cm), as well as the gradient strength (gzlvl1) and the resulting plateau width (delta).

   When the system asks “Set this value?” respond with **y**.

   The value of gcal times the appropriate value below gives the maximum achievable gradient strength.

   - Performa I: gcal × 2048.
   - Performa II/XYZ: gcal × 32767.

8. Repeat the experiment with gzlvl1 set to one of the following. The delta should be equal to that previously measured.

   - Performa I: **gzlvl1=–250**
   - Performa II/XYZ: **gzlvl1=–4000**

X and Y Axes PFG Profile for Performa XYZ

Run this tests for XYZ probes and Performa XYZ PFG amplifiers.

1. Use the parameters from the Z-axis profile experiment.
2. Create the gradaxis parameter if it does not already exist:

   create('gradaxis','string') su

   Pulsing the X and Y gradients with the profile pulse sequence and parameter set requires the gradaxis parameter.
3. Set **gradaxis='x' gzlvl1=8000**. Enter **ga** to acquire a spectrum.
4. Enter **ga**. When acquisition finishes, enter **f full dc vsadj**.

   You should see an elliptical profile, about 10 kHz wide, as shown in Figure 4.

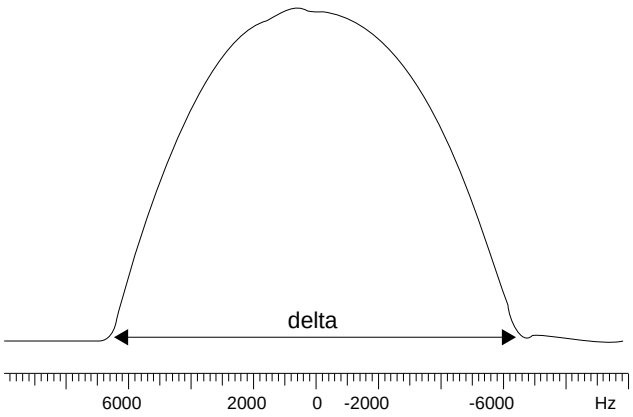


Figure 4. Transverse-Gradient Profile Measurement Using 1% Doped H_2O

5. Use the cursors to find the width of this peak at 0% of the height.
6. Enter **setgcal** to convert the width of this plateau (delta) and the gradient strength (gzlvl1) to a gradient strength per DAC value (gcal).

   The setgcal macro needs the physical extent of the sample region (0.42 cm), as well as the gradient strength (gzlvl1) and the resulting plateau width (delta).

   When the system asks “Set this value?” respond with **y**.

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The value of `gcal` times the appropriate value below gives the maximum achievable gradient strength.

- Performa XYZ: `gcal` × `32767`.

7. Repeat the experiment with **`gzlvl1=-8000`**. The `delta` should be equal to that previously measured.
8. Set **`gradaxis='y' gzlvl1=8000`**. Enter **`ga`** to acquire a spectrum. Repeat steps 4 to 7. Again, note all maximum gradient strength values for future reference. The profile for the Y gradient also looks like that shown in Figure 4.

Z Axis PFG Recovery for Performa I,II, and XYZ

1. Using **`mp`**, move the doped D_2O parameters to a new experiment. Join (**`jexp`**) the experiment.
2. Enter **`rtp('/vnmr/tests/grecovery')`**.
3. On *Performa XYZ systems*, create the `gradaxis` parameter if it does not already exist:

   ```
   create('gradaxis','string')
   gradaxis='z' su
   ```

4. Set **`tpwr`** and **`pw`** for the 90 pulse width.
5. Set the appropriate recovery array (refer to Specifications for Triple-Resonance Probes, page 18 for recovery specifications):
   - Probes with 1 ms recovery specification: enter **`array('d2',30,.001,.001)`**
   - Probes with 50 µs recovery specification: enter **`array('d2',30,.00005,.00005)`**
6. Enter **`d2=d2,1`** to add a 1-second reference spectrum.
7. Enter the appropriate value of `gzlvl1`:
   - Performa I: **`gzlvl1=10/gcal`** to change `gzlvl1` to a value that equals 10 G/cm.
   - Performa II/XYZ: **`gzlvl1=30/gcal`** to change `gzlvl1` to a value that equals 30 G/cm.
8. Enter **`ga`** to start the acquisition.
9. Enter **`wft ds(arraydim)`**.
10. Phase the final spectrum, adjust its vertical scale, and enter **`dssh`**.

    The resulting profile should show a smooth and monotonic recovery pattern, similar to Figure 5.

X and Y Axis PFG Recovery for Performa XYZ

Run this tests for XYZ probes and Performa XYZ PFG amplifiers.

1. Use the parameters from the Z-axis recovery experiment.
2. Create the `gradaxis` parameter if it does not already exist:

   ```
   create('gradaxis','string') su
   ```

   Pulsing the X and Y gradients with the `profile` pulse sequence and parameter set requires the `gradaxis` parameter.
3. Set **`tpwr`** and **`pw`** for the 90 pulse width.

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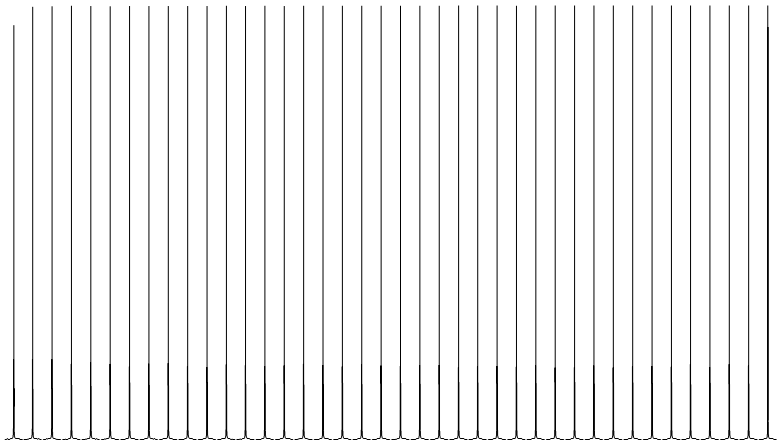


Figure 5. Gradient Recovery Pattern Profile

4. Set the following recovery array:

   `array('d2',30,.001,.001)`
5. Enter **`d2=d2,1`** to add a 1-second reference spectrum.
6. Enter **`gradaxis='x' gzlvl1=32767,-32767`**.
7. Enter **`ga`** to start the acquisition.
8. Enter **`wft ds(arraydim)`**.
9. Phase the final spectrum, adjust its vertical scale, and enter **`dssh`**.

   The resulting profile should show a recovery pattern, similar to Figure 5, which is smooth and monotonic.
10. Enter **`gradaxis='y'`** and repeat steps 7 to 9.

^{1}H Sensitivity

1. Enter **`rtp('/vnmr/tests/h1sn') su`**.
2. Tune the probe with the appropriate sample inserted.
3. Determine the pw90 on this sample using the procedure in ^{1}H Observe PW90 and RF Homogeneity, page 6.

   You can also determine the pw90 from the pw360 and dividing that value by 4.
4. Enter **`ga`** to acquire the spectrum.
5. When the spectrum is displayed, phase it and display the quartet, using the region from the right of the aromatic peak to the right of the quartet (see Figure 6).
6. Use the cursors to locate the best 200-Hz noise region to the left (downfield) of the quartet.
7. Enter **`dsnmax(200)`**. The computer calculates the S/N ratio. Answer **`'y'`** to plot the results.
8. Write the results for each probe in the forms provided in Specifications for Triple-Resonance Probes, page 18

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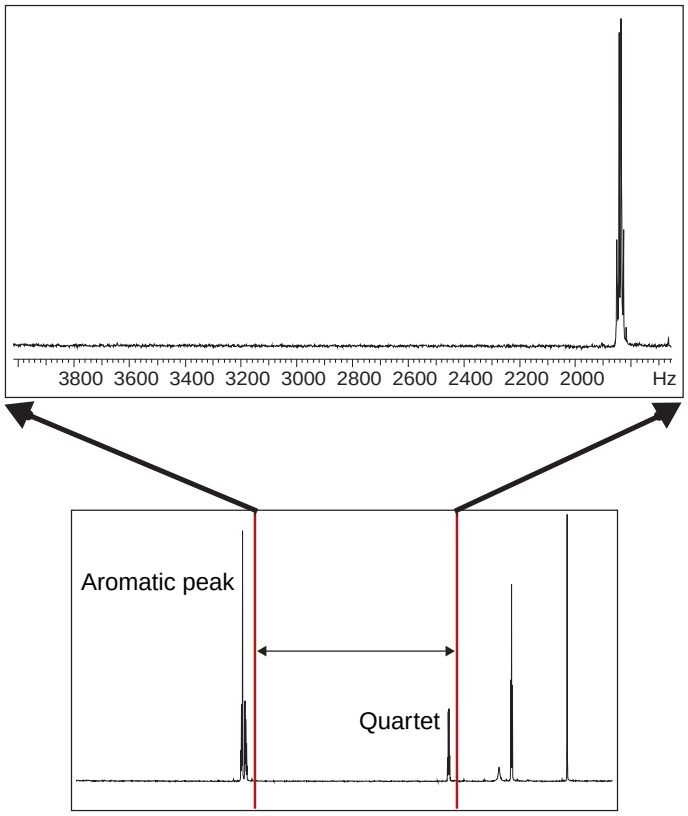


Figure 6. ^{1}H Sensitivity Measurement

^{13}C pwx90 Pulse Width and RF Homogeneity

1. Check that the ^{1}H quarter-wavelength cable is installed 400- MHz systems), the appropriate filters for ^{1}H and ^{13}C installed, and the probe is tuned for ^{1}H observe on channel 1 and ^{13}C on channel 2 using the appropriate sample listed above.
2. Enter **`rtp('/vnmr/tests/c13pwcal')`** to retrieve the test parameter set to the current experiment.
3. Change **`pw`** to the ^{1}H 90° pulse width (determined from the previous section). Change **`tpwr`** to a `tpwr` level to give the ^{1}H 90° pulse width. Acquire a normal ^{1}H spectrum of the 1% iodomethane-^{13}C, 1% trimethyl phosphite, 0.2% Cr(acac) in $CDCl_3$ sample.

   The large three-line pattern (1:1:1 should be centered approximately in the spectrum) in the spectrum at about 2.2 ppm are the peaks of interest. Place the cursor on the center peak of the three-line pattern, and enter **`nl movetof`**.
4. Enter **`pwxcal`** to set up the parameters for the ^{13}C rf homogeneity test.

   The `pwxcal` pulse sequence allows for the indirect observation of the X nuclei of interest. To use channel 2 (decoupler 1) for ^{13}C, answer the questions as follows:

```
Use decoupler 1, 2, or 3? 1
Calibrate C13, N15, or P31? C13
```


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5. Enter the appropriate **dof** and **j** values, as listed in the following table:

Parameter	*^{1}H frequency (MHz)*	*^{13}C (Hz)*	*^{15}N (Hz)*	*^{31}P (Hz)*
dof	800	-22600	1200	18500
dof	750	-21000	1100	17500
dof	600	–17000	700	14000
dof	500	–14500	0	12000
dof	400	–11000	–12000	9000
dof	300	–9000	–9200	7000
j	all	151	86	20

6. Determine the X-nucleus 90° pulse width and rf homogeneity by arraying **pwx1**:
   a. Enter **r1=*xx*/10**, where *xx* is the specified pwx90 value for the probe.
   b. Enter **array('pwx1',86,0,r1)** to create a pwx1 array covering approximately 720 degrees of nutation.

   For example, if a probe has a pwx90 specification of 15 µs, following the above protocol sets up the experiment with an array if pwx1 from 0 µs to 127.5 µs, in 1.5-µs increments. The maximum positive peaks occur at 0°, 360°, and 720° pulses. A 90° pulse gives the first null in the pwx1 array of spectra.
7. Enter **ga** to start the acquisition.
8. After the last spectrum finishes, enter **ds(1)** to display the first spectrum.
9. Enter **aph0** to phase the spectrum, and then enter **vsadj ai dssh** to display the arrayed spectra.
10. Enter **ds(1) full** to display only the single spectrum. Move the cursor to one of the ^{13}C enriched peaks of the iodomethane (one of the outer peaks of the three-peak pattern). Determine the amplitude by entering **nl** and mark to display the height of the peak (in mm) and the frequency of the peak (in Hz).

    Record the peak height (in mm) corresponding to the 360° pulse width spectrum. Repeat the steps for the spectra corresponding to 360° and the 720° pulse width spectra, respectively. Determine the peak amplitudes for the 360° pulse width and the 720° pulse width spectra. Record the peak heights (in mm) corresponding to the 360° pulse width and the 720° pulse width spectra.
11. Calculate the amplitude ratios as a percentage (e.g., (360°/0° ratio)(100) equals the rf homogeneity specification) for the 360°/0° and the 720°/0°. Plot the arrayed spectra. Record the probe type, probe serial number, and the 360°/0° and 720°/0° percentages on the form in Specifications for Triple-Resonance Probes, page 18.

^{15}N pwx90 Pulse Width and RF Homogeneity

1. Check that the ^{1}H quarter-wavelength cable is installed, the appropriate filters for ^{1}H and ^{15}N installed, and the probe is tuned for ^{1}H observe on channel 1 and ^{15}N on channel 2 (or channel 3 if applicable) using the appropriate sample listed above.
2. Enter **rtp('/vnmr/tests/n15pwxcal')** to retrieve the test parameter set to the current experiment.
3. Change **pw** to the ^{1}H 90° pulse width (determined from the ^{1}H rf homogeneity section). Change **tpwr** to the tpwr level to give the ^{1}H 90° pulse width.

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4. Acquire a normal ^{1}H spectrum of the 2% benzamide-^{15}N, 0.2% Cr(acac) in DMSO-d_6 sample.

   The peaks of interest are the benzamide multiplet at the far left side of the spectrum.

5. Move the cursor to the center of the multiplet and enter **`movetof`**.

6. Enter **`pwxcal`** to set up the parameters for the ^{15}N rf homogeneity test.

   The `pwxcal` pulse sequence allows indirect observation of the X nuclei of interest.

   To use channel 2 (decoupler 1) for ^{15}N, answer the questions as follows:

   ```
   Use decoupler 1, 2, or 3? 1
   Calibrate C13, N15, or P31? N15
   ```

   After the `pwxcal` for ^{31}P has been run using `dec1`, the pwx90 can be determined using the remaining channels by adjusting the parameters as shown:

   - If the system has third channel rf, channel 3 (decoupler 2) can be used by entering `2` in response to the first question.
   - If decoupler 2 is used for ^{15}N, use the appropriate parameters (`dpwr2`, `dn2`, `dof2`, etc.) for decoupler 2.

7. Enter the appropriate values of **`dof`** (or `dof2`—for decoupler 2) and **`j`**, as listed in the following table:

Parameter	*^{1}H frequency (MHz)*	*^{13}C (Hz)*	*^{15}N (Hz)*	*^{31}P (Hz)*
`dof`	600	–17000	700	14000
`dof`	500	–14500	0	12000
`dof`	400	–11000	–12000	9000
`dof`	300	–9000	–9200	7000
`j`	all	151	86	20

8. Determine the X-nucleus 90° pulse width and rf homogeneity by arraying **`pwx1`**:

   a. Enter **`r1=`*`xx`*`/10`**, where *xx* is the specified `pwx90` value for the probe.

   b. Enter **`array('pwx1',86,0,r1)`** to create a `pwx1` array covering approximately 720 degrees of nutation.

   For example, if a probe has a pwx90 specification of 36 µs, following the above protocol sets up the experiment with an array if `pwx1` from 0 µs to 306 µs, in 3.6-µs increments. The maximum positive peaks occur at 0°, 360°, and 720° pulses. A 90° pulse gives the first null in the `pwx1` array of spectra.

9. Enter **`ga`** to start the acquisition.

10. After the last spectrum finishes, enter **`ds(1)`** to display the first spectrum.

11. Enter **`aph0`** to phase the spectrum, and then enter **`vsadj ai dssh`** to display the arrayed spectra.

12. Enter **`ds(1) full`** to display only the single spectrum. Move the cursor to one of the ^{13}C enriched peaks of the iodomethane (one of the outer peaks of the three-peak pattern). Determine the amplitude by entering **`nl`** and `mark` to display the height of the peak (in mm) and the frequency of the peak (in Hz).

    Record the peak height (in mm) corresponding to the 360° pulse width spectrum. Repeat the steps for the spectra corresponding to 360° and the 720° pulse width spectra, respectively. Determine the peak amplitudes for the 360° pulse width and

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the 720° pulse width spectra. Record the peak heights (in mm) corresponding to the 360° pulse width and the 720° pulse width spectra.

13. Calculate the amplitude ratios as a percentage (e.g., (360°/0° ratio)(100) equals the rf homogeneity specification) for the 360°/0° and the 720°/0°. Plot the arrayed spectra. Record the probe type, probe serial number, and the 360°/0° and 720°/0° percentages on the form in Specifications for Triple-Resonance Probes, page 18.

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Specifications for Triple-Resonance Probes

Specifications are listed for the following probes:

- 400-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ PFG Triple-Resonance, page 19
- 500-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ PFG triple resonance, page 20
- 500-MHz 8-mm $^{1}H\{^{13}C/^{15}N\}$ PFG Triple-Resonance, page 21
- 500-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ XYZ PFG Triple-Resonance, page 22
- 600-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ PFG triple resonance, page 23
- 600-MHz 8-mm $^{1}H\{^{13}C/^{15}N\}$ PFG Triple-Resonance, page 24
- 600-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ XYZ PFG Triple-Resonance, page 25

All specifications are subject to change without notice. The specifications published in this document shall prevail unless customer contract determines otherwise.

Request of any additional specifications beyond those listed in this document must be agreed upon in writing as part of the customer contract.

Specifications are for probes installed with currently-shipping Varian UNITY*INOVA* NMR spectrometers. For probes purchased for use in systems other than currently-shipping UNITY*INOVA* spectrometers, no guarantee is given that these probes meet current specifications.

Tests are performed with the following sample tubes:

- 3-mm probes: 3-mm tubes with 0.30-mm wall (Wilmad 327-PP, or equivalent).
- 5-mm probes: 5-mm tubes with 0.38-mm wall (Wilmad 528-PP, or equivalent).
- 8-mm probes: 8-mm tubes with 0.51-mm wall (Wilmad 513A-7PP, or equivalent).
- 10-mm probes: 10-mm tubes with 0.46-mm wall (Wilmad 513-7PP, or equivalent).
- *Using sample tubes with thinner wall thickness (e.g., Wilmad 5-mm 545-PPT, or equivalent; Wilmad 10-mm 513-7PPT, or equivalent) increases signal-to-noise.*

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400-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ PFG Triple-Resonance

Probe Serial No. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	15		
^{15}N pwx	45		

- RF Homogeneity

Nucleus	*Spec. (%)*	*Results*
^{1}H (450/90)	80	
^{1}H (810/90)	70	
^{13}C (360/0)	70	
^{13}C (720/0)	55	
^{15}N (360/0)	70	
^{15}N (720/0)	50	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Nonspinning ($CHCl_3$)*	*Results*
14-, 18-shim	0.45/6.0/12.0	—	
23-shim	0.45/6.0/12.0	0.65/10.0/20.0	

- ^{1}H Spinning Sidebands

Nucleus	*Spec. (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	450:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa I	>18	≤ 1ms @ 10 gauss/cm	
Performa II/XYZ	>65	≤ 50 µs @ 30 gauss/cm	

- Variable Temperature Range
  –20 to +80 C

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500-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ PFG triple resonance

Probe serial no. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	15		
^{15}N pwx	45		

- RF Homogeneity

Nucleus	*Specification (%)*	*Results*
^{1}H (450/90)	80	
^{1}H (810/90)	70	
^{13}C (360/0)	70	
^{13}C (720/0)	55	
^{15}N (360/0)	70	
^{15}N (720/0)	50	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Non-spinning ($CHCl_3$)*	*Results*
23-shim	0.45/6.0/12.0	0.65/10.0/20.0	
28-shim	0.45/5.0/10.0	0.65/8.0/16.0	
Ultra-shim	0.45/5.0/10.0	0.65/6.0/12.0	

- ^{1}H Spinning Sidebands

Nucleus	*Specification (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	800:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa I	>18	≤ 1ms @ 10 gauss/cm	
Performa II/XYZ	>60	≤ 50 µs @ 30 gauss/cm	

- Variable Temperature Range
  –20 to +80 C

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500-MHz 8-mm $^{1}H\{^{13}C/^{15}N\}$ PFG Triple-Resonance

Probe Serial No. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	20		
^{15}N pwx	50		

- RF Homogeneity

Nucleus	*Spec. (%)*	*Results*
^{1}H (450/90)	70	
^{1}H (810/90)	50	
^{13}C (360/0)	70	
^{13}C (720/0)	40	
^{15}N (360/0)	70	
^{15}N (720/0)	40	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Nonspinning ($CHCl_3$)*	*Results*
28-shim	0.45/5.0/10.0	0.65/8.0/16.0	
Ultra-shim	0.45/5.0/10.0	0.65/8.0/16.0	

- ^{1}H Spinning Sidebands

Nucleus	*Spec. (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	1150:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa I	>18	≤ 1ms @ 10 gauss/cm	
Performa II/XYZ	>65	≤ 1ms @ 30 gauss/cm	

- Variable Temperature Range
  –20 to +80 C

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500-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ XYZ PFG Triple-Resonance

Probe Serial No. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	15		
^{15}N pwx	45		

- RF Homogeneity

Nucleus	*Spec. (%)*	*Results*
^{1}H (450/90)	80	
^{1}H (810/90)	70	
^{13}C (360/0)	70	
^{13}C (720/0)	55	
^{15}N (360/0)	70	
^{15}N (720/0)	50	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Nonspinning ($CHCl_3$)*	*Results*
23-shim	0.45/6.0/12.0	0.65/10.0/20.0	
28-shim	0.45/5.0/10.0	0.65/8.0/16.0	
Ultra-shim	0.45/5.0/10.0	0.65/6.0/12.0	

- ^{1}H Spinning Sidebands

Nucleus	*Spec. (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	800:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa XYZ, Z axis	>65	≤ 1 ms @ 30 gauss/cm	
X axis	>25	≤ 2 ms @ 25 gauss/cm	
Y axis	>25	≤ 2 ms @ 25 gauss/cm	

- Variable Temperature Range
  0 to +50 C

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600-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ PFG triple resonance

Probe serial no. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	15		
^{15}N pwx	45		

- RF Homogeneity

Nucleus	*Specification (%)*	*Results*
^{1}H (450/90)	80	
^{1}H (810/90)	70	
^{13}C (360/0)	70	
^{13}C (720/0)	55	
^{15}N (360/0)	70	
^{15}N (720/0)	50	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Non-spinning ($CHCl_3$)*	*Results*
23-shim	0.45/6.0/12.0	0.65/10.0/20.0	
28-shim	0.45/5.0/10.0	0.65/8.0/16.0	
Ultra-shim	0.45/5.0/10.0	0.65/6.0/12.0	

- ^{1}H Spinning Sidebands

Nucleus	*Specification (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	1000:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa I	>18	≤ 1 ms @ 10 gauss/cm	
Performa II/XYZ	>60	≤ 50 µs @ 30 gauss/cm	

- Variable Temperature Range
  –20 to +80 C

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600-MHz 8-mm $^{1}H\{^{13}C/^{15}N\}$ PFG Triple-Resonance

Probe Serial No. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	20		
^{15}N pwx	50		

- RF Homogeneity

Nucleus	*Spec. (%)*	*Results*
^{1}H (450/90)	70	
^{1}H (810/90)	50	
^{13}C (360/0)	70	
^{13}C (720/0)	40	
^{15}N (360/0)	70	
^{15}N (720/0)	40	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Nonspinning ($CHCl_3$)*	*Results*
Ultra-shim	0.45/6.0/12.0	0.65/8.0/16.0	

- ^{1}H Spinning Sidebands

Nucleus	*Spec. (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	1700:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa I	>18	≤ 1 ms @ 10 gauss/cm	
Performa II/XYZ	>65	≤ 1 ms @ 30 gauss/cm	

- Variable Temperature Range
  –20 to +80 C

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600-MHz 5-mm $^{1}H\{^{13}C/^{15}N\}$ XYZ PFG Triple-Resonance

Probe Serial No. _____ Customer Initials _____

- 90° Pulse Width

Nucleus	*PW90 (µs)*	*Results*	*Notes, tpwr/dpwr*
^{1}H	10		
^{13}C pwx	15		
^{15}N pwx	45		

- RF Homogeneity

Nucleus	*Spec. (%)*	*Results*
^{1}H (450/90)	80	
^{1}H (810/90)	70	
^{13}C (360/0)	70	
^{13}C (720/0)	55	
^{15}N (360/0)	70	
^{15}N (720/0)	50	

- Resolution and Lineshape (50%/0.55%/0.11%)

Shim Set	*^{1}H Spinning ($CHCl_3$)*	*^{1}H Nonspinning ($CHCl_3$)*	*Results*
23-shim	0.45/6.0/12.0	0.65/10.0/20.0	
28-shim	0.45/5.0/10.0	0.65/8.0/16.0	
Ultra-shim	0.45/5.0/10.0	0.65/6.0/12.0	

- ^{1}H Spinning Sidebands

Nucleus	*Spec. (%)*	*Results*	*Notes*
^{1}H	1		

- Sensitivity

Nucleus	*(S:N)*	*Results*	*Notes*
^{1}H	1000:1		

- PFG Profile and Recovery

PFG System	*Profile*	*Recovery*	*Results*
Performa XYZ, Z axis	>65	≤ 1 ms @ 30 gauss/cm	
X axis	>25	≤ 2 ms @ 25 gauss/cm	
Y axis	>25	≤ 2 ms @ 25 gauss/cm	

- Variable Temperature Range
  0 to +50 C

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Triple-Resonance NMR Probe Installation
Varian NMR Spectrometer Systems
Pub. No. 01-999125-00, Rev. A0699

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