

BARE BONES GUIDE TO Single-Peak NOE-Difference on the Inova-400&500

These are simplified NOE-Difference Instructions when you only wish to irradiate a single peak (or multiplet). Please follow the separate instructions for Multiple-Peak NOE-difference if you wish to irradiate several peaks (or multiplets) in a single experiment.

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Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<angle brackets> are used to designate a key to be pressed (i.e. <Ret> for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

LMB will be used to designate the Left Mouse Button

MMB will be used to designate the Middle Mouse Button

RMB will be used to designate the Right Mouse Button

Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

Note: All **commands** in boldface assume that you press <Return> afterwards.

Important Considerations before starting:

- This version of *steady-state* NOE replaces the former “noediff” macro. It uses the “cyclenoe” sequence, which saturates multiplet frequencies individually, using lower power. The advantage is that selectivity of presaturation is greatly improved, and artifacts are significantly reduced. The difference *FID* is generated directly, eliminating the need for mathematical subtraction of on/off resonance FIDs, and eliminating about 90% of the usual subtraction errors.
- For optimum results, the sample temperature should be controlled. To set the temperature do the following:
 - If the Temperature Control window is not already running, type **temp**, and the temperature control window should appear. Adjust the slider to the desired temperature (21 degrees C is recommended for ambient), and wait until the sample temperature equilibrates to the desired temperature. The green LED labeled “VT” will flash until the temperature is regulated, and then should illuminate continuously.
 - To disable temperature control, *click* on “Turn Temperature Control Off”, and the green LED over “VT” should turn off. If the first click doesn’t work, click on it again. Reduce the “Temp” window to an icon, or close it using the Right Mouse Button.

- 1) Acquire a normal ^1H NMR spectrum, setting **nt=4**.
- 2) Identifying multiplet for presaturation:
 - a) Zoom-in on the multiplet that you wish to irradiate for NOE.
 - i) Place the cursor exactly at the center of the multiplet, and type **sd**. Note the frequency displayed at the top of the window, as this will be the value for satfrq for this peak.
 - ii) Carefully measure the coupling constant (or average frequency spacing) of the multiplet:
Note: if the peak you are irradiating is a singlet, set **spacing=0** and **pattern=1**.
 - (1) Use two cursors (left button/right button) to measure the frequency separation of the peaks in the multiple, type “**delta?** “. Note this value..this will be the value for spacing for this peak.
 - (2) Or you can set **axis='h'** and the spacing (in Hz) will be displayed on the screen (as *delta*).
 - iii) Note the number of peaks in this multiplet, as this will be the value for pattern for this peak.
 - (1) again, if the peak being irradiated is a singlet, you will set pattern=1 below.
 - b) Finally, select an open region of noise (not near any peaks), place the cursor there, and type **sd**. Note this frequency, as it will be used as the control parameter (off-resonance presaturation frequency).

- 3) Turn OFF the Spinner:
 - a) Type **spin=0 su** , or manually turn it off in the [Lock] window in *acqi*.
 - 4) Type **cyclenoe** to load the standard parameters.
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- 5) Type **satfrq=#**, where “#” is the value determined for *satfrq* above in Step #2
 - 6) Type **spacing=#** where “#” is the value determined for *spacing* in Step #2
 - 7) Type **pattern=#**, where “#” is the value determined for *pattern* in Step #2..
 - 8) Type **control=#**, where # is the frequency value determined for *control* in step-(2-b) above.
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- 9) Type **satpwr?**, and note the value. It should be between “-16” and “-12”. If the individual lines in your multiplet (being irradiated) are unusually broad, you might need to increase the value of *satpwr* Other parameters that you might want to check (type **dg** to display parameters). :
 - a) **sattime=4**
 - b) **d1=1, bs=8**
 - c) **ss=4** (using 8 might minimize artifacts due to incomplete T1 relaxation)
 - d) **intsub = ‘y’**
 - e) **cycle = ‘y’**
 - 10) Set NT (# transients):
 - a) Type **nt=32** (or more). The more transients you perform, the better the results will be.
 - 11) Type **time** to see how long the experiment will take.
 - 12) Type **go** to start the experiment.
 - 13) Type **lb=0.5** to minimize difference artifacts (try lb=1.0 for an even better result)
 - 14) After a block (bs) is done, you can do **wft**, and phase and manipulate as usual.
 - 15) Type **svf** , followed by your filename, to save the data. If you selected multiple irradiation frequencies, all spectra will be saved in a single “fid” file.
- 16) Quitting:
 - a) Type **jexp1** , eject your sample, and re-insert the reference.
 - b) Be sure to turn the spinner back on in [**Acqi**].
 - c) Lock and shim on the reference, and sign-out in the logbook.

Processing, Analyzing, and Plotting the NOE Difference spectrum:

- 1) Type **wft** as usual, and you can try **aph** to auto-phase; however, you will probably need to phase the spectrum manually.
- 2) Use manual phasing, to phase the irradiated peak to be perfectly negative, and the residual peaks due to NOE enhancement positive.
- 3) Integrate the peaks using the normal procedure, making sure to include the integral region that includes the negative (irradiated) peak.
- 4) Since the irradiated peak will be negative, you might wish to increase “vp”, to move the baseline up on the screen; however, the shift axis (*dscale*) will cross the negative peak. To prevent this, you can increase “vp”, and specify that the scale be drawn at the baseline.
 - a) Type **vp=60 ds**
 - b) Type **dscale(12)**
 - c) When plotting, the commands would be: **pl pscale(12) page**.
- 5) Analyzing/Quantitating the %NOE.
 - a) This can be done by using the irradiated peak as an internal standard. In the Varian software, you cannot enter a negative value for an integral. Therefore you should phase the spectrum with the irradiated peak POSITIVE, and the values of your NOEs will appear as negative numbers. By integrating the selected/saturated peak, and setting the integral value for that region ([Set Int]) to 100 (for a CH proton), or 200 (for a CH₂ signal), or 300 (for a CH₃) signal, the %NOE can be read directly by integrating the positive peaks resulting from NOE enhancement.
 - i) Example 1: If a CH₃ is irradiated, and there is an NOE to a nearby CH₂ signal, you would set the integral value (using [Set Int]) to 300.0, and measure the integral value for the negative (residual) CH₂ signal. Since there are 2 equivalent protons for the CH₂, you would divide the integral value by 2 for %NOE. (i.e. if the measured integral for the CH₂ were -30.0, after normalizing the negative CH₃ integral to 300.0, the reported NOE enhancement would be 15%).
 - ii) Example 2: If a CH₂ multiplet were irradiated, and an NOE enhancement were observed to a nearby CH resonance, you would set the integral value for the irradiated peak to 200.0, and read the integrated intensity of the residual signal from the CH proton. Since this is a single proton, the displayed integrated intensity (negative number) is a direct measure of the %NOE enhancement.

-R.Shoemaker