

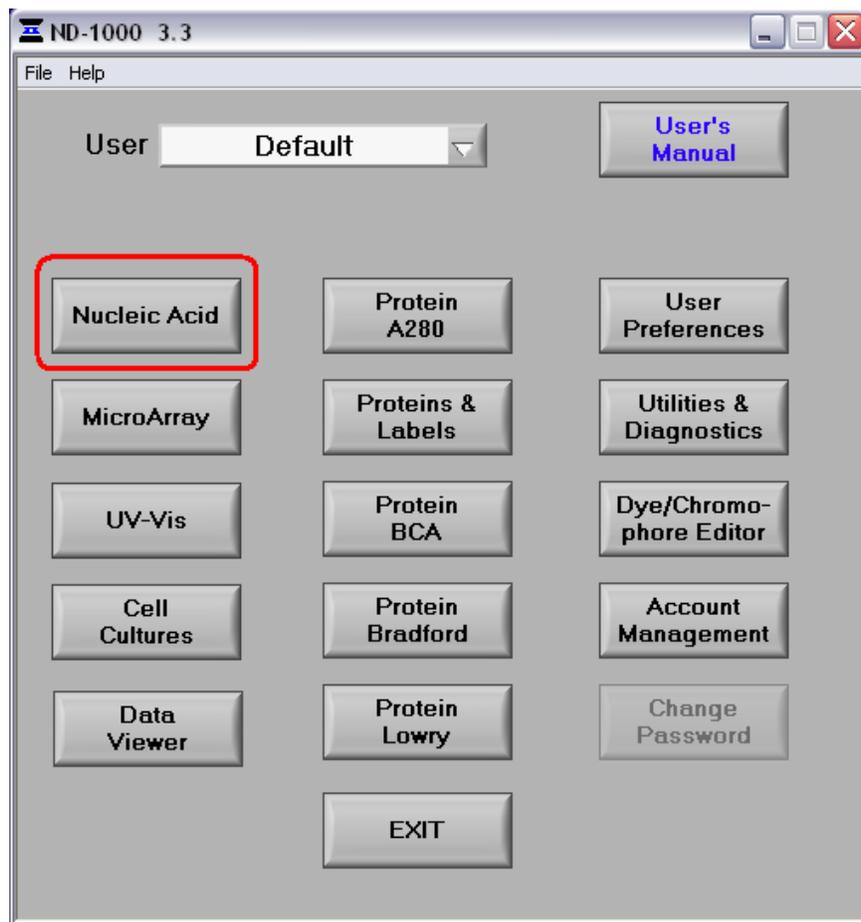
Determining the Concentration of your Morpholino in Solution using the NanoDrop ND-1000 Spectrophotometer

(Assumes ND-1000 Version 3.3.0)

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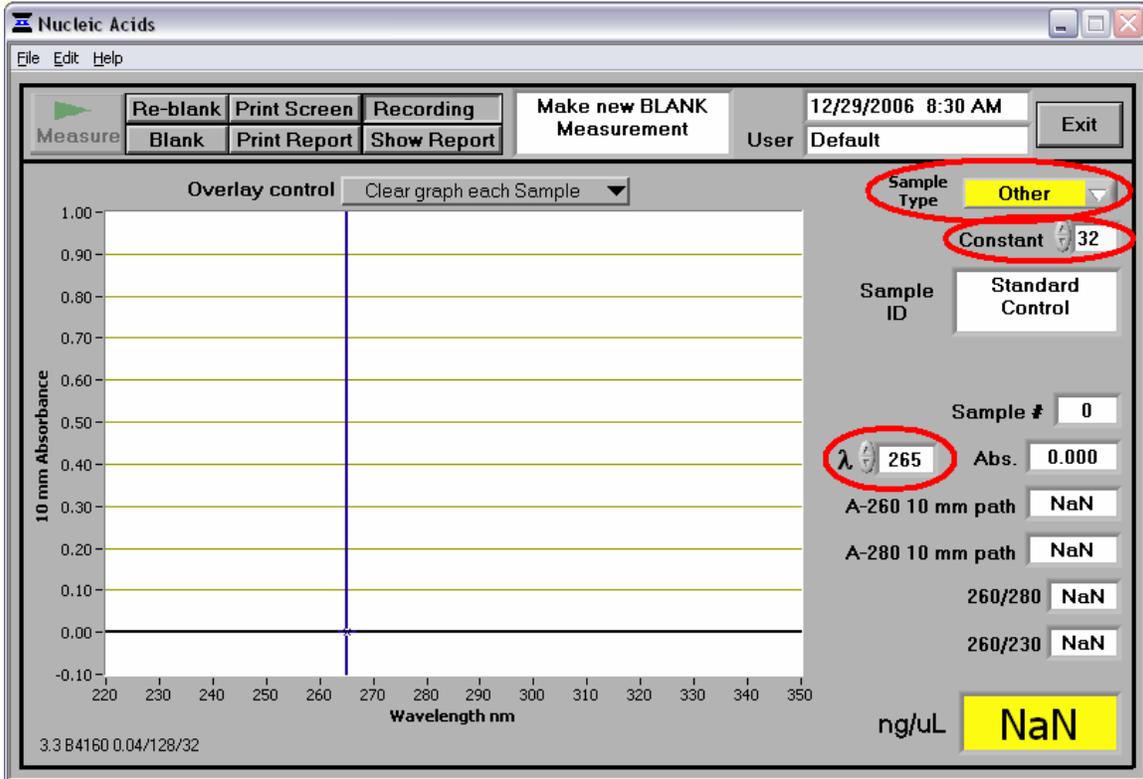
Note: The NanoDrop spectrophotometer uses a very short path length for measuring light absorbance, but the software included with the NanoDrop normalizes the data to the standard 1 cm path length typically used by most spectrophotometers.

Start the ND-1000 software, which will display the Application Modules buttons and other diagnostic and user preference buttons. (See figure below.) **Click the Nucleic Acid button.**



You will be instructed to ensure the sample pedestals are clean and to load a water sample to initialize the instrument.

Once the OK button is clicked, the following screen will be displayed.



To quantify your Morpholino solution, you will need to change three settings on this screen:

1. Change the **Sample Type** to **Other**.
2. Change the **Wavelength (λ)** from 230 to **265**.
3. Calculate and enter the appropriate **Constant** value for your Morpholino. The **Constant** is calculated using the wavelength-dependent molar absorptivity coefficient (Molar Abs) and the molecular weight (MW) of the specific Morpholino you are quantifying.

$$\text{Constant} = \text{molecular weight} * 1000 / \text{absorptivity coefficient}$$

The molar absorptivity coefficient and molecular weight information is provided on the product information sheet that was shipped with your Morpholino.

Name/Quantity	Sequence/End Mod/Production #	MW	Molar Abs.	Weight	OD	Holdback
Standard Control 1000 nmol	CCTCTTACCTCAGTTACAATTTATA 47-11Dec06C-K	8328	259160	8.33	259.16	300 nmol

For our standard control example above: The Morpholino standard control sequence 5'CCTCTTACCTCAGTTACAATTTATA 3' has molar absorptivity of 259160 and molecular weight of 8328.

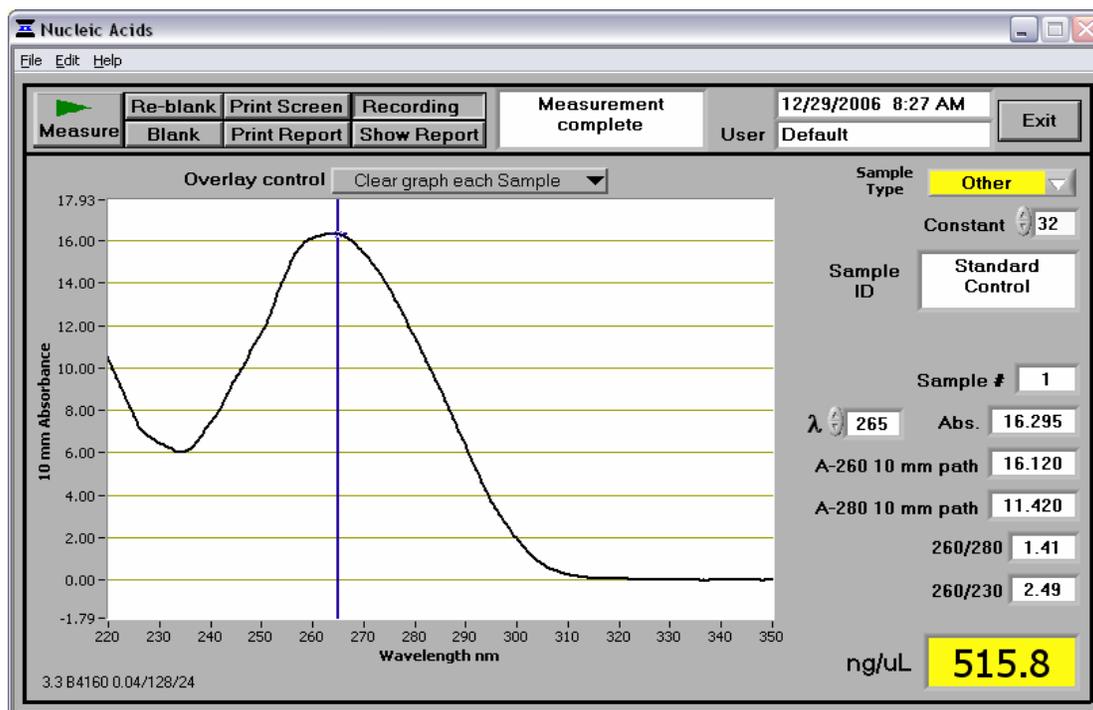
$$\text{Constant} = 8328 * 1000 / 259160 = 32$$

Note - Contact Gene Tools with your Morpholino production number if you have misplaced your product information sheet, or alternatively you can retrieve the information immediately from our website, www.gene-tools.com by going to our Online Store. Once logged onto the store, select **My Orders**, then the appropriate order number with the Morpholino for which you wish to obtain the datasheet. Click on the link **Custom Oligo Information Sheet** under the **Downloads for This Order** section and the datasheet for that order will be displayed.

Once you have changed the sample type and entered the correct wavelength and constant values, blank the spectrophotometer by loading 2 μ l of 0.1 N HCl in the spectrophotometer and clicking the **Blank** button. When the blank is finished (~10 sec), wipe the measurement pedestals clean with a soft laboratory wipe.

Dilute 5 μ l of the Morpholino solution into 95 μ l of 0.1 N HCl (20X dilution) and place 2 μ l of this solution on the measurement pedestal, click the **Measure** button.

You should get something that looks similar to the figure below.



The spectrophotometer is reporting a Morpholino concentration of 515.8 ng/μL. To calculate the concentration of your original Morpholino solution, multiply this by the dilution factor of 20X, which in this example results in a concentration of **10,320 ng/μL (or 10.32 μg/μL)**.

To calculate the millimolar concentration of your Morpholino solution, simply divide the concentration obtained above (in ng/μl) by the molecular weight of your Morpholino. In our standard control example, we find $10320 / 8341 = 1.2 \text{ mM}$ **solution**.

Update - 25 January 2007

Using the NanoDrop ND-1000 Version 3.3.0 software, please be aware that changing the wavelength (λ) does not change the calculation of the ng/μL reported at the bottom of the form in yellow. This calculation is **always** based on the absorbance at **260 nm**, regardless of the actual number in the wavelength box.

Fortunately in the case of Morpholinos, the peak of the absorbance curve is broad and flat enough to only impact the result slightly when comparing the calculations at 260 nm and 265 nm. If you would like to have a more accurate calculation of the oligo concentration, simply multiply the absorbance reading given at 265 nm (found to the right of the wavelength (λ) entry) times the constant you calculated for your Morpholino using its molar absorptivity coefficient and molecular weight information.

For our example above, the concentration displayed by the software using the fixed 260 nm wavelength gave us 515.8 ng/μL and a final concentration of 10.32 ug/μL. If we correct for the reading at 265 nm instead of 260 nm by using the absorbance reported as 16.295, then multiplied by the constant of 32 appropriate for this Morpholino, we get 521.44 ng/μL or a final concentration of 10.43 ng/μL. Note that there is only a 1% error between the results at 260 nm and 265 nm.