

# How to Use Guide for the Nanodrop Spectrophotometer

-Open Nanodrop Software

-Choose application (for this guide we will use "Nucleotides")

-Lift arm over pedestal and apply 2µl of RNase free water to the pedestal, close arm lightly, and hit "OK" to initialize.

-Choose DNA or RNA

-Lift arm over pedestal, wipe water off pedestal and arm with a dry kimwipe, apply 2µl of blank solution (whatever your nucleotides are resuspended in), and gently close arm.

-Hit "Blank" and the machine will blank the machine.

-When it displays a flat graph and a quantitation near 0, lift arm over pedestal, wipe blank off pedestal and arm with a dry kimwipe, apply 2µl of sample solution, and gently close arm.

-Enter Sample ID. Upper right button in middle of screen should say "Recording" (If it says record, hit it and it should switch to recording).

-Hit "Measure" and the machine will measure your sample.

-The program will give you:

- a quantitation in ng/µl
- a graph of the absorbance
- 260/280 ratio
- 260/230 ratio

The graph and ratios should be used to determine the purity, and thus, the accuracy of the quantitation. As absorbance measurements will measure any molecules absorbing at a specific wavelength, nucleic acid samples will require purification prior to measurement to ensure accurate results. Nucleotides, RNA, ssDNA, and dsDNA all will absorb at 260 nm and contribute to the total absorbance.

## 260/280

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

Some researchers encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to the NanoDrop® ND-1000 spectrophotometer. The three main explanations for this observation are listed below:

## CHANGE IN SAMPLE ACIDITY

Small changes in the pH of the solution will cause the 260/280 to vary\*. Acidic solutions will under-represent the 260/280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3. If comparing the ND-1000 or ND-8000 spectrophotometer to other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on the ND-1000 or ND-8000 is at the same pH and ionic strength as the diluted sample measured on the second spectrophotometer.

\* William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, *Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity*: *BioTechniques* 22:474-481 (March 1997)

## WAVELENGTH ACCURACY OF THE SPECTROPHOTOMETERS

Although the absorbance of a nucleic acid at 260 nm is generally on a plateau, the absorbance curve at 280 nm is quite steeply sloped. A slight shift in wavelength accuracy will have a large effect on 260/280 ratios. For example, a +/- 1 nm shift in wavelength accuracy will result in a +/- 0.2 change in the 260/280 ratio. Since many spectrophotometers claim a 1 nm accuracy specification, it is possible to see as much as a 0.4 difference in the 260/280 ratio when measuring the same nucleic acid sample on two spectrophotometers that are both within wavelength accuracy specification.

## **NUCLEOTIDE MIX IN YOUR SAMPLE**

*The five nucleotides that comprise DNA and RNA exhibit widely varying 260/280 ratios. The following represent the 260/280 ratios estimated for each nucleotide if measured independently:*

*Guanine: 1.15*

*Adenine: 4.50*

*Cytosine: 1.51*

*Uracil: 4.00*

*Thymine: 1.47*

*The resultant 260:280 ratio for the nucleic acid being studied will be approximately equal to the weighted average of the 260/280 ratios for the four nucleotides present. It is important to note that the generally accepted ratios of 1.8 and 2.0 for DNA and RNA respectively, are "rules of thumb". The actual ratio will depend on the composition of the nucleic acid. Note: RNA will typically have a higher 260/280 ratio due to the higher ratio of Uracil compared to that of Thymine.*

### **260/230**

*This ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for "pure" nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230nm.*

*A typical spectral pattern for Nucleic Acid starts around 8.00 at 220nm, drops 2-3 points as it reaches 230nm, then has a gradual bell curve, peaking at 260nm and falling to 0 by about 300-310nm*

*EDTA carbohydrates and phenol all have absorbance near 230 nm. The TRIzol reagent is a phenolic solution which absorbs in the UV both at 230 nm and ~270 nm.*

*Guanidine HCL used for DNA isolations will absorb at ~230 nm while guanidine isothiocyanate, used for RNA isolations will absorb at ~260 nm.*

**All samples should be measured in duplicate (triplicate if results are wildly different). When done, open your report. If you need to change a sample name, select the line affected, hit 'Data' 'Rename' and rename it. When ready, hit 'Reports' 'Save Report', usually choose 'Export Report Table only' and then open the resulting .txt file in excel. Can also view the "Plots" tab at the top of the report, hover over the legend button to get all plots labeled, and hit PrintScreen and paste into Paint and save so you have a single, concise plot for all samples.**