Specific NanoDrop Instrument Recommendations to Achieve Accurate Oligonucleotide Quantification Results

Brian Matlock, Thermo Fisher Scientific, Wilmington, DE, USA

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Introduction

The quantification of nucleic acids has traditionally been performed by absorbance measurements at 260 nm. Researchers will often use absorbance measurements as a quality control step for various applications involving nucleic acid molecules including oligonucleotides, dsDNA, and RNA. When quantifying oligonucleotides with Thermo Scientific™ NanoDrop™ instrumentation, special considerations are needed to ensure the most accurate quantification result. These considerations relate directly to the unique structural characteristics of oligonucleotides and the specific measurement parameters that the NanoDrop microvolume instrumentation uses for quantification of these small molecules. Our aim in this technical note is to provide our users with specific recommendations to help them get the most accurate oligonucleotide quantification result using NanoDrop instruments.

Oligonucleotide Characteristics that can Affect the Absorbance Quantification Result

Most molecular biologists are very familiar with the UV spectrum of nucleic acid molecules. The distinct features of a nucleic acid spectrum are easy to identify: a prominent peak at 260 nm and the characteristic trough at 230 nm (Figure 1). However, it is important to remember that each individual nucleotide has its own unique absorbance profile (λ_{max} for all bases is between 255 nm and 280 nm) and that the classic nucleic acids' UV spectrum is actually the combined spectra of each individual nucleotide. The UV spectrum and extinction coefficients of synthetic oligonucleotides can vary widely from that exhibited by other nucleic acid molecules. Because oligonucleotides are short, single-stranded molecules, their UV spectrum and extinction coefficient will be more closely dependent on base composition and sequence context than that of DNA or RNA. Therefore, to ensure the most accurate quantification results for oligonucleotides, it is critical to use an oligo-specific conversion factor rather than the general ssDNA conversion factor of 33 µg/OD_{260} (Table 1). This can be done by selecting either custom or oligo from the sample type options in the software.

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Oligo Sequence	Oligo Specific Conversion factor (µg/A_{260})	A_{260}	Concentration using general ssDNA conversion factor 33 (ng/µL)	Concentration using Oligo specific coefficient (ng/µL)	% Difference
AAA AAA AAA AAA AAA AAA	25.41	20.75	684.75	527.26	26.0
/56-FAM/CCC CCT TTT CCC CCT CCC TTT CCC CCT CCC TTT CCC CCT TTT CCC	38.18	36.96	1219.68	1411.13	14.6
CTC AAT TGT AGG TAC TAC TTC	32.19	19.97	659.01	642.83	2.5

Table 1: Effect of not using oligo-specific conversion factors to determine the concentration of various constructs. It is not uncommon to see quantification results to be off by 5–10%. In extreme cases, however, the quantification result can be off by up to 26%.

Various modifications, such as fluorophores, are often placed on either the 5' or 3' ends of oligonucleotides. Many of these modifications will absorb light in the UV or visible regions of the spectrum and can affect the quantification result. To get the most accurate quantification result, it is important to know and take into account the A_{260} correction factor for the modification. The easiest way to ensure that the appropriate correction factor is used when calculating the concentration of an oligo is by using the Thermo Scientific MicroArray module of the NanoDrop software. The MicroArray module includes options that will perform this type of correction automatically.

The traditional purity ratios (260/280 and 260/230), which are used as an indication of the presence of various contaminants in nucleic acid samples, do not apply for oligonucleotides because the shape of their UV spectrum is highly dependent on their base composition (Figure 1).

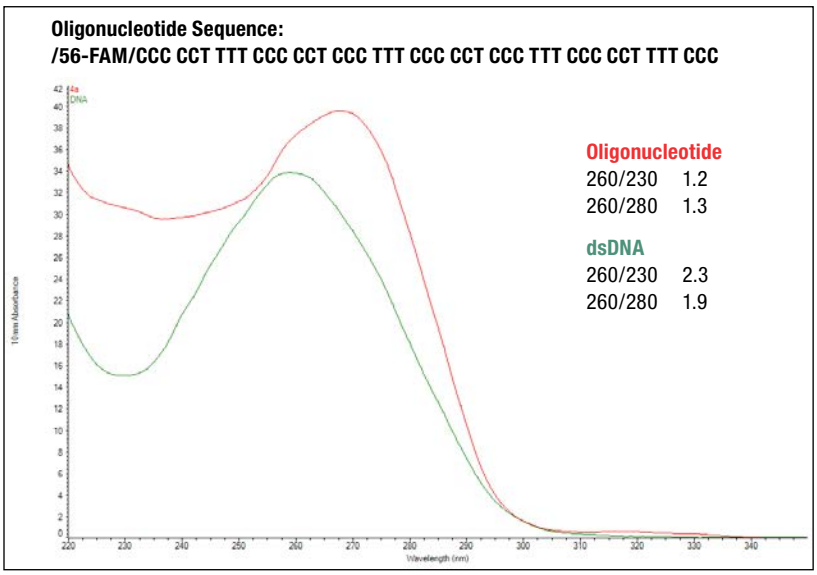

Figure 1: The UV spectrum of a pure oligonucleotide (red). As shown here, the purity ratios can deviate significantly from the accepted values for large nucleic acid molecules.

NanoDrop-specific Considerations will Ensure an Accurate Determination of Oligonucleotide Concentration

There are instrument-specific considerations that need to be taken into account when validating oligonucleotide concentrations with a NanoDrop instrument. Following these recommendations will ensure the most accurate concentration result. NanoDrop instruments can measure a wide concentration range because of their capability to read samples using multiple pathlengths (Figure 2). It is important to note that the instrument's acceptable error increases as the pathlength is shortened. In many instances, the concentration of an oligonucleotide stock will be high enough for the instrument to use these very short pathlengths. Therefore, we recommend making dilutions to ensure that measurements are being made with the 1 mm or 0.2 mm pathlengths, which will provide the most accurate oligonucleotide quantification result. The 1 mm or 0.2 mm optical pathlengths have much smaller error tolerances, 3% and 5% respectively.

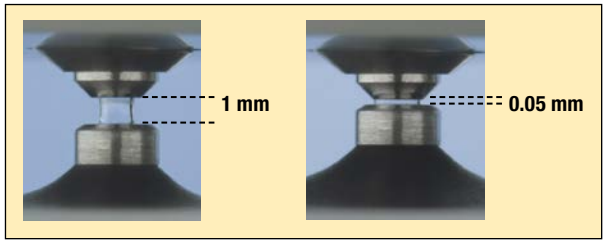

Figure 2: The Thermo Scientific NanoDrop 2000 can take measurements at pathlengths that range from 1 mm to 0.05 mm. This enables the instrument to measure a wide range of nucleic acid concentrations. It is important to realize that the error tolerances of the shorter pathlengths are higher than those of the longer pathlengths.

To determine the approximate absorbance of your oligonucleotide stock, you can use the Beer-Lambert equation shown below (Figure 3 and 4). To ensure that the absorbance is being measured by using either the 1 mm or 0.2 mm pathlength, it is important to avoid measuring over 62.5A at 260 nm.

Another consideration that needs to be taken into account is the baseline correction. NanoDrop's default setting is to perform baseline correction at 340 nm. In most cases, it is important to have this correction performed because it corrects for any light scattering events that may skew the result. However, in some cases, a more accurate result will be obtained when the baseline correction is turned off. For example, if an oligonucleotide has a modification that will absorb light at 340 nm, the baseline correction should be turned off. In this case, the researcher does not want the absorbance at 340 nm to be removed from the A_{260} value because doing so will produce an incorrect result.

$$A = \varepsilon bc$$

Where:

A = Absorbance (1 cm)

ε = Molar Extinction Coefficient (L/(mole cm))

b = Pathlength

c = Concentration (M, mole/L)

Figure 3: The Beer-Lambert equation can be used to determine the theoretical absorbance of your oligonucleotide stock. Most oligonucleotide manufactures will provide the oligo's molar extinction coefficient, which can be used in this equation.

Oligonucleotide Extinction Coefficient = 227200 (L/(mole cm))

Oligonucleotide Stock Concentration = 100 µM

Where:

A_{260} = 227200 * (1 cm) * (0.0001 M)

A_{260} = 22.72

Figure 4: Example on how to use the Beer-Lambert equation to determine the absorbance of a 100 µM oligonucleotide stock. This example shows that to obtain the A_{260} for a 100 µM solution you can simply divide the oligo's extinction coefficient by 1000.

Conclusion

In the modern molecular biology laboratory, Thermo Scientific NanoDrop instruments have become a common tool to QC various types of nucleic acid molecules including oligonucleotides, dsDNA and RNA. In this paper, we have summarized NanoDrop-specific considerations that must be taken into account when measuring the concentration of oligonucleotides. The following considerations will ensure the most accurate quantification result: (1) using an oligo-specific conversion factor rather than the general ssDNA conversion factor of 33 µg/OD_{260}; (2) when measuring oligonucleotides with modifications using the MicroArray module of the NanoDrop software, which has options that automatically perform this type of correction; (3) making dilutions to ensure that measurements are being made with the 1 mm or 0.2 mm pathlengths; and (4) turning off the default baseline correction if an oligonucleotide has a modification that will absorb light at 340 nm.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
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