Accurate oligonucleotide quantification using Thermo Scientific™ NanoDrop™ instruments

This technical note is the result of collaboration between Application Scientists at IDT and Thermo-Fisher.

Factors affecting absorbance quantification

Quantification of nucleic acids has traditionally been performed by absorbance measurements at 260 nm. Researchers often use absorbance measurements as a quality control step for various applications involving nucleic acid molecules, including oligonucleotides, dsDNA, and RNA. To obtain the most accurate oligonucleotide quantification result when using Thermo Scientific™ NanoDrop™ instruments, it is important to take into account a couple factors. These factors relate directly to the unique structural characteristics of your oligonucleotide(s) and the specific measurement parameters that the NanoDrop microvolume instrument uses for quantification of these small molecules. This article describes our recommendations for how to take these factors into account.

Oligonucleotide characteristics contributing to absorbance quantification results

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).

Oligonucleotide sequence:

/56-FAM/CCC CCT TTT CCC CCT CCC TTT CCC CCT CCC TTT CCC CCT TTT CCC

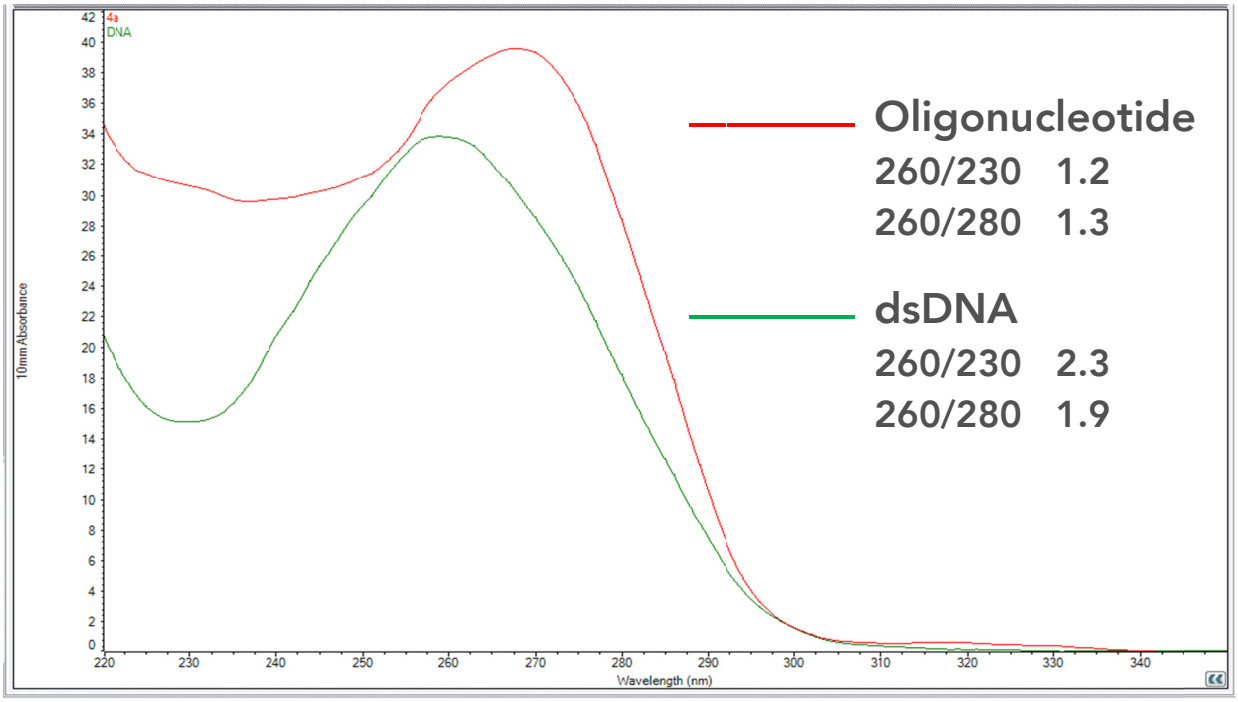

Figure 1. UV spectrum of a pure oligonucleotide (red). As shown here, the purity ratios for a short oligonucleotide can deviate significantly from the accepted values for large nucleic acid molecules.

However, it is important to remember that each individual nucleotide has its own unique absorbance profile (λ_{max} for all bases is between 255–280 nm), and that the overall nucleic acid UV spectrum is actually the combined spectra of each individual nucleotide within the sequence. The UV spectra and extinction coefficients of synthetic oligonucleotides can vary widely from those exhibited by other forms of nucleic acid molecules. Because oligonucleotides are short, single-stranded molecules, their UV spectra and extinction coefficients are more closely dependent on base composition and sequence context than for longer, single-stranded DNA or RNA, or for or double-stranded molecules. To ensure you obtain the most accurate quantification results for your 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). You can do this by selecting either **custom** or **oligo** from the **sample type** options in the NanoDrop™ software.

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: Failure to use an oligo-specific conversion factor can affect oligonucleotide concentration readings by >5%. It is not uncommon to see quantification results that are off by 5–10% when the wrong conversation factor is used. In extreme cases, the quantification result can be off by >25%.

Various modifications, such as fluorophores, are often placed on 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 quantification results. To get the most accurate quantification result, it is important for you to take into account the A_{260} correction factor for these modifications. The easiest way to incorporate the appropriate correction factor when calculating the concentration of an oligo is by using the MicroArray module of the NanoDrop software. The MicroArray module provides options that perform this type of correction automatically.

Traditional purity ratios ($A_{260/280}$ and $A_{260/230}$), used as an indication of the presence of various contaminants in nucleic acid samples, also do not apply for oligonucleotides because the shape of the oligonucleotide UV spectrum is highly dependent on base composition (Figure 1).

NanoDrop-specific considerations ensure accurate oligonucleotide concentration

There are a few instrument-specific factors to take into account when validating oligonucleotide concentrations with a NanoDrop instrument. By following these recommendations you will ensure the most accurate concentration result.

Path length. NanoDrop instruments can measure a wide concentration range because of their capability to read samples using multiple path lengths (Figure 2). It is important to note that the instrument's acceptable error increases as the path length is shortened. In many instances, the concentration of an oligonucleotide stock will be high enough for the instrument to use these very short path lengths. However, we recommend diluting these concentrated stock solutions to ensure you make your measurements using the 1 mm or 0.2 mm path lengths. These path lengths provide the most accurate oligonucleotide quantification, since they have much smaller error tolerances—3% and 5%, respectively.

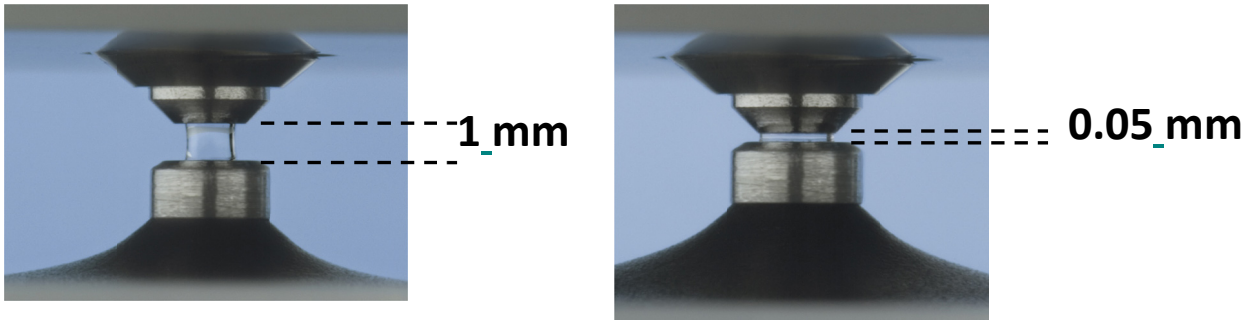

Figure 2. Path length affects error tolerance. The NanoDrop 2000 can take measurements at path lengths of 1–0.05 mm. This enables the instrument to measure a wide range of nucleic acid concentrations. It is important to realize that error tolerances for shorter path lengths are higher than those for longer path lengths.

Determine the approximate absorbance of your oligonucleotide stock using the Beer-Lambert equation (Figures 3 and 4). You can ensure measurement of absorbance at 1 mm or 0.2 mm path length by avoiding measurement of absorbance values above 62.5 at 260 nm.
To achieve the most accurate quantification results with the NanoDrop, dilute oligonucleotide stocks to obtain absorbance <12.5. Alternatively, you can rearrange the Beer-Lambert equation to determine the expected concentration threshold for a given path length. By measuring a concentration lower than this calculated value, you will ensure use of the longer path length.

$$A = \varepsilon\, b\, c$$

Where:

A = Absorbance

ε = Molar attenuation coefficient (L/(mole·cm))

b = Path length (cm)

c = concentration (M, mole/L)

Figure 3. The Beer-Lambert Equation. Use this equation to determine the theoretical absorbance of your oligonucleotide stock. Most oligonucleotide manufacturers will provide the oligo molar extinction coefficients for use in this equation.

Example:

Oligonucleotide attenuation coefficient =227,200 (L/(mole·cm))

Oligonucleotide stock concentration = 100 µM

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

A_{260} = 22.72

Figure 4. Using the Beer-Lambert Equation to determine absorbance. This example shows how to obtain the A_{260} for a 100 µM oligonucleotide stock solution. Simply divide the oligo extinction coefficient by 10,000.

Example:

Oligonucleotide attenuation coefficient = 227,200 (L/(mole·cm))

Oligonucleotide stock concentration = 100 µM

c = 62.5 / (227200 x (1 cm))

c = 0.000275 M = 275 µM

Figure 5. Using the Beer-Lambert Equation to determine concentration threshold for a specific path length.

Baseline correction. You must also take baseline correction into account. The default setting of the NanoDrop instruments is to perform baseline correction at 340 nm. In most cases it is important to have this correction performed because it adjusts for any light scattering events that may skew results. However, in some cases, you may obtain a more accurate result when the baseline correction is turned off. For example, if an oligonucleotide includes modification that absorbs light at 340 nm, the baseline correction should be turned off. In this case, you do not want absorbance at 340 nm to be removed from the A_{260} value as doing so will produce an incorrect result.

Summary of absorbance measurement adjustments

In the modern molecular biology laboratory, NanoDrop instruments have become a common tool for performing QC of various types of nucleic acid molecules, including oligonucleotides, dsDNA, and RNA. However, you need to include oligonucleotide-specific and NanoDrop instrument–specific adjustments to optimize quantification results:

(1) Use an oligo-specific conversion factor instead of the general ssDNA conversion factor of 33 µg/OD_{260}.
(2) When measuring oligonucleotides with modifications, use the MicroArray module of the NanoDrop software. It provides options that automatically correct for modification absorbance.
(3) Make dilutions to ensure absorbance measurements use 1 mm or 0.2 mm path lengths.
(4) Turn off the default baseline correction for oligonucleotides with modifications that absorb light at 340 nm.