

UV/Visible Spectroscopy



The Quantification of Oligonucleotide Using a LAMBDA 465 UV/Vis Spectrophotometer

Introduction

The efficiency of molecular manipulations involving nucleic acids is heavily dependent on the concentration and purity of the nucleic acid in a sample. The

quantification of oligonucleotide is readily accomplished by taking advantage of the absorbance of UV light. Thus, UV light analysis may be used to derive information about the concentration of the sample. This application note describes the quantification of an oligonucleotide using a LAMBDA™ 465 UV/Vis Spectrophotometer.

Principle

To quantify the amount of oligonucleotide, readings should be taken at wavelengths of 260 nm and 280 nm. The reading at 260 nm allows the calculation of the concentration of nucleic acid in the sample. An A_{260} of 1 corresponds to approximately 25 $\mu\text{g/ml}$ for oligonucleotide.

This measurement permits the direct calculation of the concentration in a sample:

$$[\text{Oligonucleotide}] \mu\text{g/ml} = A_{260} \times 25 \times \text{dilution}$$

Where, A_{260} = absorbance at 260 nm

dilution = dilution factor

25 = extinction coefficient of oligonucleotide

Reagents and Apparatus

Oligonucleotide (23 mer)

Deionized water

LAMBDA 465 UV/Vis Spectrophotometer

UV Lab software

Cuvettes (10 mm pathlength, Semi-micro cell)

Procedure

1. Dissolve oligonucleotide in deionized water.
2. Dilute the sample serially to give a range of concentrations.
3. Select Quantification Standard in the view bar.
4. Instrument parameters are as follows:
(see Fig.1)

Experiment Setup

Data type: Absorbance

Sampling: Single cell

Mode: Faster (Spectra No. : 1, Scan No. : 10,
Integration No. : 1, Gain No. : 1)

Experiment Type: Quantification Standard

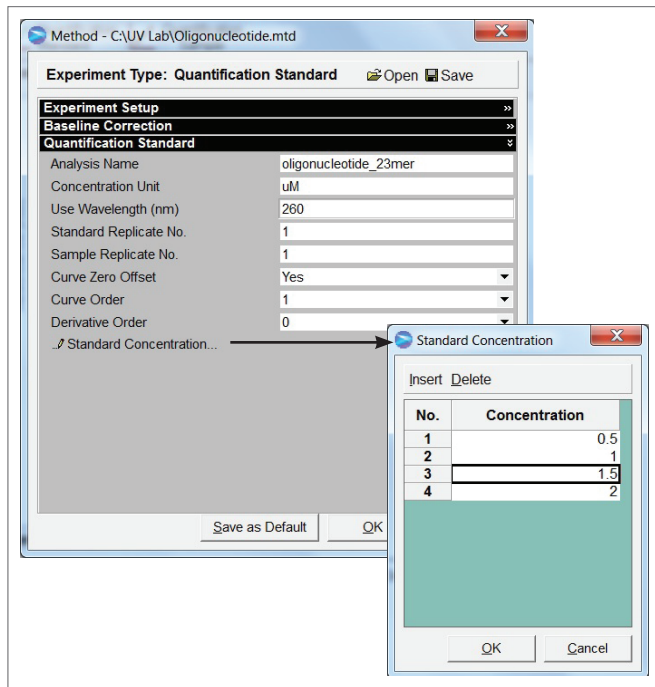


Figure 1. Experimental Setup for quantification of oligonucleotide.

Result

1. Concentration of oligonucleotide

Table 1. gives the results of the A_{260} value and concentration. Figure 2 and Figure 3 show the spectra and standard curve.

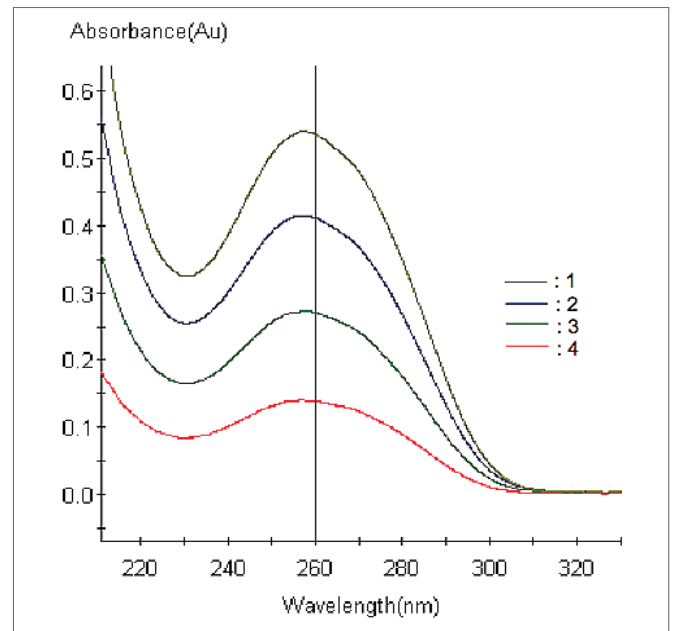


Figure 2. Spectra of oligonucleotide.

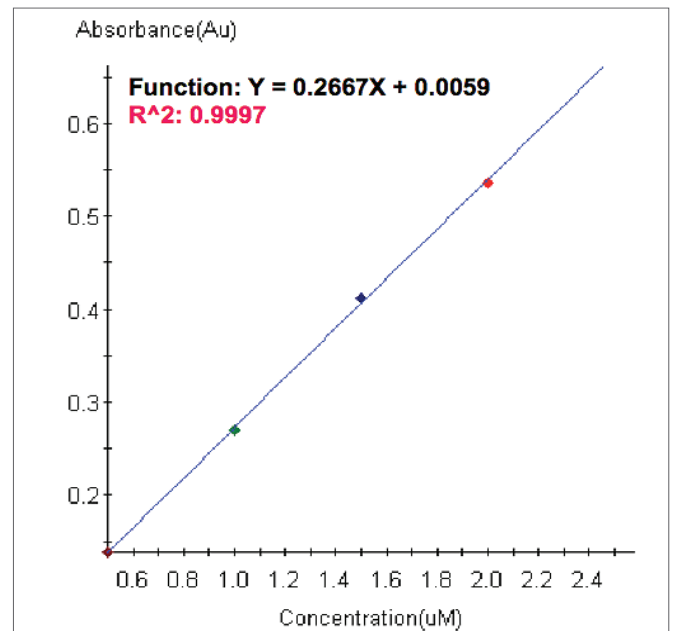


Figure 3. Standard curve of oligonucleotide.

Table 1. Concentration and A_{260} value.

Spectrum No.	Conc. (μM)	Au (260 nm)
4	0.5	0.1387
3	1.0	0.2704
2	1.5	0.4120
1	2.0	0.5360

Conclusion

Using the LAMBDA 465 UV/Vis Spectrophotometer and UV Lab software, quantification of the oligonucleotide was performed. The range of concentrations was 0.5 - 2.0 μ M. Rapid acquisition of spectra and good sensitivity were obtained with the LAMBDA 465. The UV Lab™ software was used to perform the quantitative analysis and to process the data efficiently.

References

1. Maniatis, F.L., Fritsch, E.F., Sambrook, J., Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, New York, 1982.
2. Robert E. Farrell, Jr., RNA Methodologies: Academic Press, 1993.