UV/VIS Excellence Line

DNA Analysis

UV/VIS Spectrophotometer Application

Quality and quantity determination of a DNA sample can be crucial for the success of downstream analysis and, in most molecular biology labs, is typically carried out by UV/Vis spectroscopy. But what information can we retrieve from the spectrum? This article describes which spectral features reveal the most valuable information about the sample.

For DNA analysis it is typically advised to scan the complete spectral range from 230 to 320 nm. Within this range, the wavelengths of most interest are 260, 280, 230 and 320 nm respectively (see Figure 1).

Absorbance at 260 nm

DNA has its absorbance maximum at 260 nm. In a pure sample, DNA concentration is proportional to absorbance. When measured in a 10 mm cell, the concentration of double-stranded DNA is approximated as 50 µg/mL per 1 absorbance unit, using the average extinction coefficient of 0.020 $(\mu g/mL)^{-1}$ cm^{-1}.

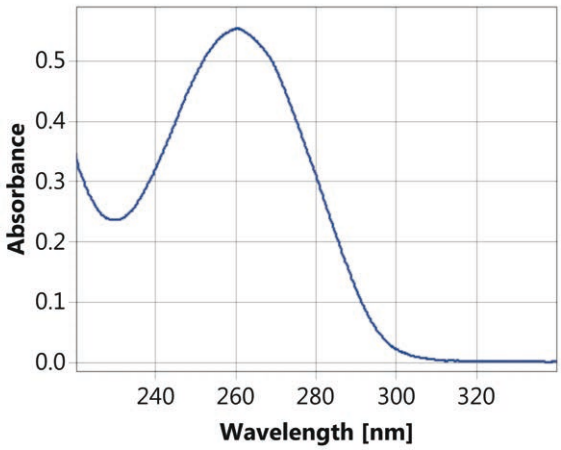

Figure 1.
Absorbance spectrum of DNA.

Single-stranded DNA concentration is approximated as 33 µg/mL per 1 absorbance unit.

However, for short oligo-nucleotide sequences, these values are not very accurate as the absorbance of each base is different. A better result can be achieved by calculating the unique extinction coefficient for each oligo-sequence. Modern software programs include an oligo-sequence calculator for determining the unique extinction coefficient.

When taking a reading at 260 nm, the presence of contaminants that also absorb at 260 nm can lead to an overestimation of DNA content. Therefore, a background correction at 320 nm is often applied. At this wavelength, a pure solution will have no absorbance; therefore the presence of contaminants, such as phenol or chloroform, will cause a higher absorbance reading.

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For the background correction, the absorbance measured at 320 nm is subtracted from the absorbance at 260 nm to provide the corrected value. This corrected value is then used to calculate the concentration.

Absorbance ratio 260/280

As proteins have their absorbance maximum at 280 nm, the ratio of absorbance at 260 and 280 nm is a good indicator of protein contamination. A ratio of 1.8 or higher indicates a pure DNA sample. If the ratio is smaller, the sample is probably contaminated by protein. As with concentration determination at 260 nm, a background correction at 320 nm can be applied.

Absorbance ratio 260/230

Additional purity information can be derived from the ratio of absorbance at 260 and 230 nm. Among the contaminants that absorb at 230 nm are organic compounds or chaotropic agents such as Urea, Guanidin-HCl, EDTA, carbohydrates and phenols. For pure DNA, the absorbance ratio 260/230 should be greater than 1.8.

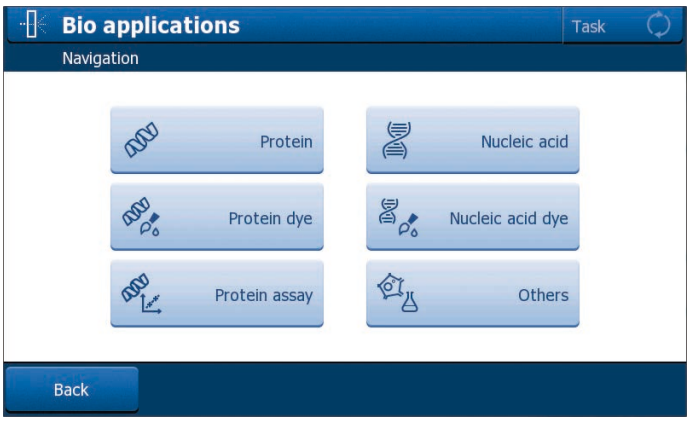

Figure 2.
The UV5 Nano includes predefined methods for DNA and protein analysis.

Summary

Knowing the correct concentration and purity of a DNA sample is crucial for downstream analysis. Using poor quality DNA or the wrong concentration can lead to incorrect results, meaning the analysis has to be repeated, wasting time and money.

UV/VIS analysis of DNA using a micro-volume spectrophotometer such as the UV5Nano is a very simple, fast and cost-efficient method of obtaining quality and quantity information from your sample. Using the predefined methods (Figure 2), the test for DNA concentration and purity is quickly and simply performed and guarantees the quality and accuracy of the results.

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