

DR6000 for Quantitative and Qualitative Determination of DNA and RNA

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Introduction

Photometric analyses for quantitative and qualitative measurement of DNA and RNA

The purification of DNA/RNA samples in molecular biology laboratories—with kits that are usually purchased—ultimately requires a check of the purity of the DNA/RNA contained therein, as contamination with proteins or organic components cannot be precluded.

A convenient method for determining the purity of DNA/RNA is photometric measurement, as it can be carried out more quickly than options like gel electrophoresis.

In addition to determining the purity of DNA/RNA, simple methods for quantifying DNA/RNA samples using UV photometry are also possible with the DR6000.

The following measurements can be taken with the DR6000:

- Determination of concentration of ssDNA/dsDNA and RNA (measurement at 260 nm)
- Determination of cleanliness of DNA/RNA – contamination with proteins (260/280 ratio)
- Determination of cleanliness of DNA/RNA – contamination with proteins and organic components such as phenolates or isocyanates (230/260/280 ratio)

Methods

Determination of concentration of ssDNA, dsDNA and RNA

The determination of the concentration of DNA or RNA is based on the absorption of the samples at 260 nm. The aromatic rings of the bases in the DNA/RNA are responsible for the absorption.

In the DR6000, the concentration is determined in a 1-cm quartz cuvette.

The DR6000 is set to zero at a wavelength of 260 nm using a cuvette containing only the solution in which the DNA/RNA (usually the TRIS buffer) is present.

The DNA/RNA solution is then measured and the concentration is determined on the basis of the measured extinction at 260 nm.

The literature describes the following conversion factors that can be used to calculate the concentration of ssDNA, dsDNA or RNA using a 1-cm cuvette on the basis of the absorption at 260 nm ($A_{260\text{ nm}}$):

$A_{260\text{ nm}}$ (1-cm cuvette) = 1.000, corresponds to

50 µg/mL dsDNA
40 µg/mL ssDNA
33 µg/mL RNA

These conversion factors are derived from the spectral absorption coefficients of ssDNA, dsDNA or RNA and the conversion of the Beer-Lambert law

Three examples of concentration measurements in the **user program DNA/RNA conc.** using purified DNA/RNA samples with increasing contents are set out below. The results are output as $\mu\text{g/L}$ dsDNA, ssDNA and RNA and can be adapted to suit requirements.

The figure shows three screenshots of the DR6000 user program interface for the '9000 DNA/RNA conc.' program, all set to UV 260 nm. Each screen displays a table of results for dsDNA, ssDNA, and RNA, along with navigation buttons (Main Menu, Zero, Read, Options) and a vertical menu (Login, Sample ID, Timer, Dilution, AQA).

9000 DNA/RNA conc. UV 260 nm		
15.8	$\mu\text{g/mL}$	dsDNA
10.4	$\mu\text{g/mL}$	ssDNA
12.6	$\mu\text{g/mL}$	RNA

9000 DNA/RNA conc. UV 260 nm		
28.5	$\mu\text{g/mL}$	dsDNA
18.8	$\mu\text{g/mL}$	ssDNA
22.8	$\mu\text{g/mL}$	RNA

9000 DNA/RNA conc. UV 260 nm		
42.2	$\mu\text{g/mL}$	dsDNA
27.8	$\mu\text{g/mL}$	ssDNA
33.8	$\mu\text{g/mL}$	RNA

Investigation of the purity of the DNA/RNA sample:

The DNA/RNA solutions obtained following purification in the laboratory are primarily contaminated with proteins or other organic components.

As proteins are absorbed at 280 nm due to the aromatic amino acid residues, the contamination of the DNA/RNA sample with proteins can be determined by measuring at 260 nm and also at 280 nm.

- A pure DNA sample has an absorption 260/absorption 280 ratio of 1.8 or greater
- A pure RNA sample has an absorption 260/absorption 280 ratio of 2.0 or greater

Additional contamination of the DNA/RNA sample may be present with organic substances such as phenolates or isocyanates. An additional measurement is taken at 230 nm to measure this contamination.

- A pure DNA sample has an absorption 230/absorption 260/absorption 280 ratio of 1/1.8/1
- A pure RNA sample has an absorption 230/absorption 260/absorption 280 ratio of 1/2/1

In the DR6000, the purity of the DNA/RNA sample can be measured by taking a simultaneous measurement of the absorption at 260 nm and 280 nm (230 nm) in a 1-cm quartz cuvette. The following figures show the results on the DR6000 following the measurement of an almost pure DNA sample and one contaminated with proteins (bovine serum albumin; BSA).





Figure 1: Result for an almost pure DNA sample (75 µg/L DNA)

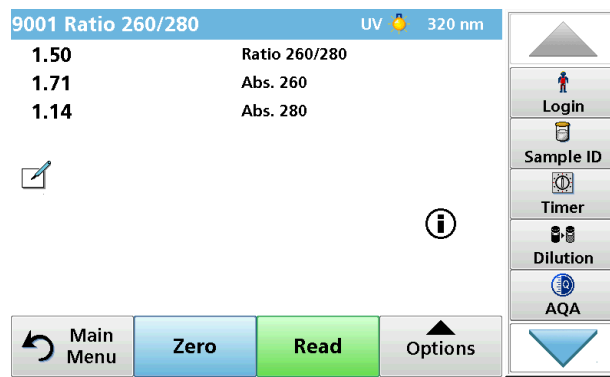


Figure 2: Result for same DNA sample contaminated with 500 µg/L BSA)

The degree of purity of the sample drops from 1.73 to 1.50 as a result of contamination with BSA. The slightly elevated absorption at 260 nm (from 1.52 absorption to 1.71 absorption) simulates an increased DNA content.

Wavelength scan

The wavelength scan function of the DR6000 can also be used to determine the purity of a DNA/RNA sample and to determine possible contamination with proteins.

The following screenshot shows wavelength scans comparing a 60 µg/L DNA sample (orange) and a 2000 µg/L BSA (bovine serum albumin) sample (blue).

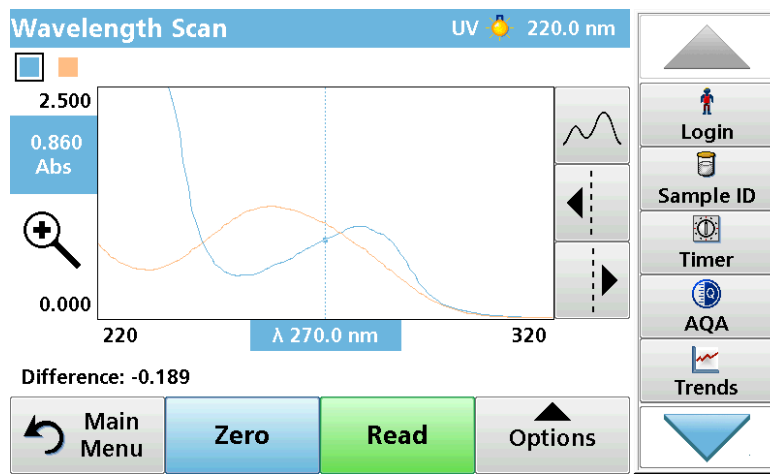


Figure 3: Wavelength scan of an almost pure DNA sample and a protein solution (BSA)

While the DNA sample shows the maximum absorption at 260 nm and the absorption at 260 nm is also significantly greater than at 280 nm, the absorption curve for the protein solution (here: 2000 µg/L BSA) shows the maximum absorption at 280 nm.

This difference in absorption behaviors for DNA and protein solutions can be used to check the purity of the DNA sample with a scan.

The figure below shows the scan for a DNA sample (60 µg/L) contaminated with protein solution (2000 µg/L) compared with an almost pure DNA sample.

It is clear that the absorption at 280 nm increases disproportionately as a result of the protein solution. The absorption 260 nm/absorption 280 nm quotient for determining the purity of the DNA sample is reduced from 1.76 to 1.19.

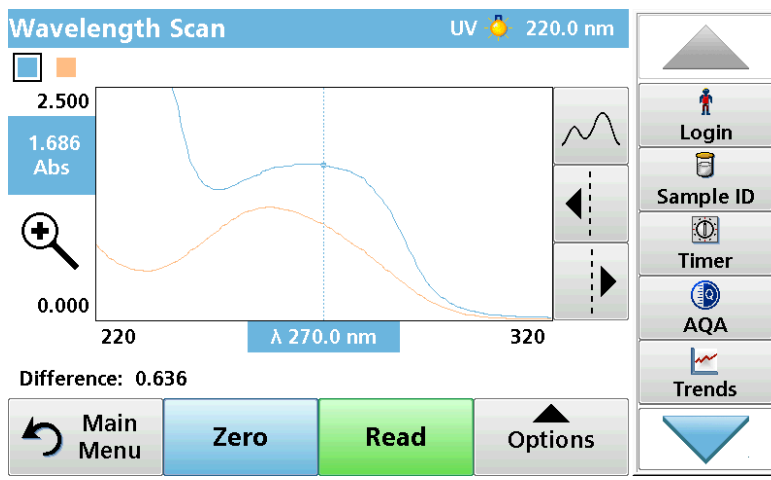


Figure 4: Absorption spectrum of a pure DNA sample (orange, reference) and one contaminated with 2000 µg/L BSA (blue)

Finally, Figure 5 shows the absorption spectra of a pure DNA sample and a DNA sample contaminated with increasing concentrations of BSA.

The shift in the spectra with increasing BSA concentrations is clearly identifiable.

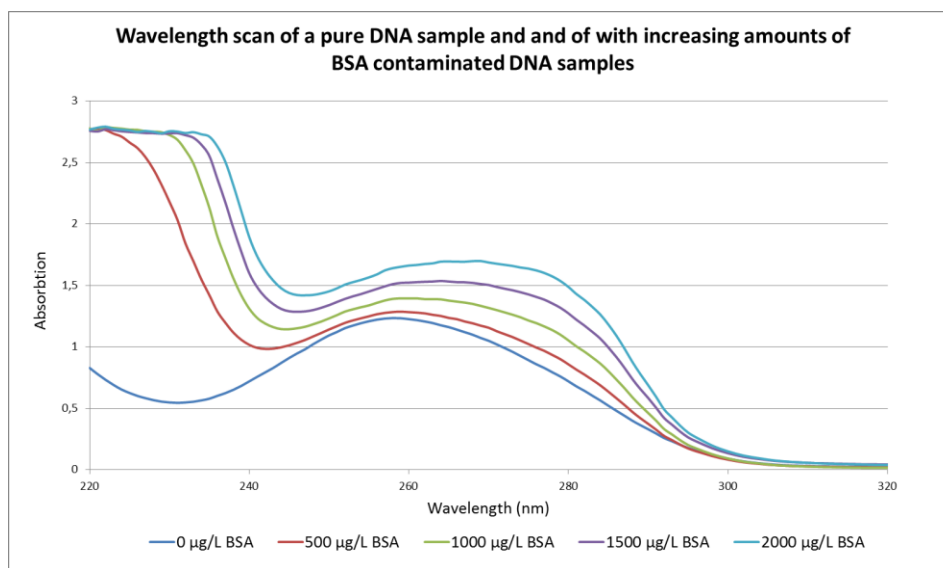


Figure 5: Absorption spectra of a pure DNA sample (0 µg/L BSA; dark blue) and DNA samples contaminated with increasing concentrations of BSA (500 – 2000 µg/L)

Summary

Its functions and options for measuring in the UV range and the wavelength scan function with a comparison against a reference make the DR6000 an important tool in the analysis of DNA and RNA samples.

Both the quantification of DNA and RNA samples and the determination of the purity of the samples can be performed quickly and easily using UV spectroscopy.

This allows the methods of the DR6000 to be adapted to the tasks of the lab as effectively as possible with freely programmable user programs.

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