

Superior linearity and reproducibility using the Agilent Cary 60 for routine measurements of micro volumes

Technical overview



Introduction

The challenge of measuring micro volume samples has become increasingly relevant as the demand for sample throughput increases, and costs per analysis are driven down. These samples typically include DNA, RNA and proteins, which absorb light in the ultraviolet region of the spectrum, typically requiring the use of expensive quartz cuvettes. Often, routine quantitative and qualitative measurements are taken before sensitive downstream applications in which sample purity and concentration accuracy are vital.

The Agilent solution for these types of measurements is the Cary 60 UV-Vis spectrophotometer with an ultra-microvolume cuvette. This paper will demonstrate the linearity and reproducibility of the Cary 60 with the ultra-microvolume cuvette for DNA concentrations less than 25 ng/ μ L.



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Materials

- Cary 60 UV-Vis spectrophotometer
- Ultra-microvolume cuvette, 1 mm path length lid
- Human Genomic DNA (Promega)
- Nuclease-free distilled water

Instrumentation/method

An ultra-microvolume cuvette was placed in a Cary 60 UV-Vis and aligned to optimize the light throughput. A drop of sample was placed on the accessory and ten measurements were taken (Figure 1). This was repeated with a commercially available instrument designed for measuring micro volumes. DNA samples were diluted to 21.7, 10.8, 5.4, 2.7, 1.35 and 0.67 ng/ μ L using nuclease-free water. In addition, eight repeated measurements of the same 21.7 ng/ μ L sample were taken to determine instrument reproducibility.



Figure 1. The ultra-microvolume cuvette for the Cary 60 is easy-to-use and easy to clean. Simply drop a sample onto the platform, replace the reflective lid, and take a measurement.

Results

A plot of measured and calculated DNA concentration highlights the linearity of the Cary 60 with the ultra-microvolume cuvette (Figure 2). Many spectrophotometers do not have the sensitivity to measure low concentrations of nucleic acids, for example, less than 25 ng/ μ L (equivalent Abs of 0.04). The performance capabilities of the spectrophotometer become vital at these low concentrations particularly for micro volume measurements where the path length used is reduced. When the sample is of a very low concentration, it is vital to perform a full wavelength scan to ensure that a typical DNA curve can be observed, rather than just instrument noise.

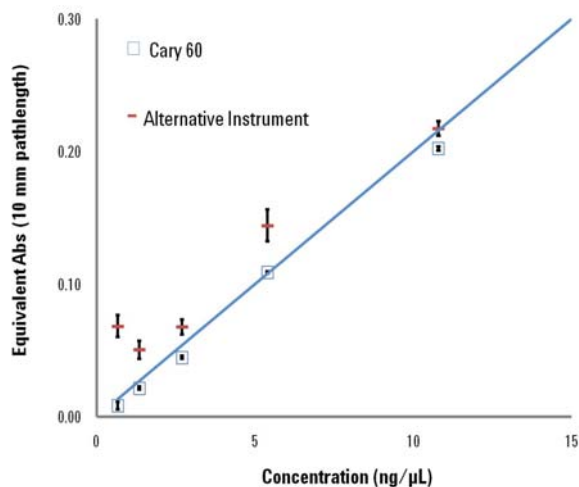


Figure 2. Linearity at low DNA concentrations. Error bars show the standard deviations of ten measurements at each concentration. The R^2 for the Cary measurements was 0.9943.

Eight repeated measurements were taken on the same DNA sample without removing the sample, to assess the reproducibility of the data obtained with the Cary 60 with the ultra-microvolume cuvette (Figure 3).

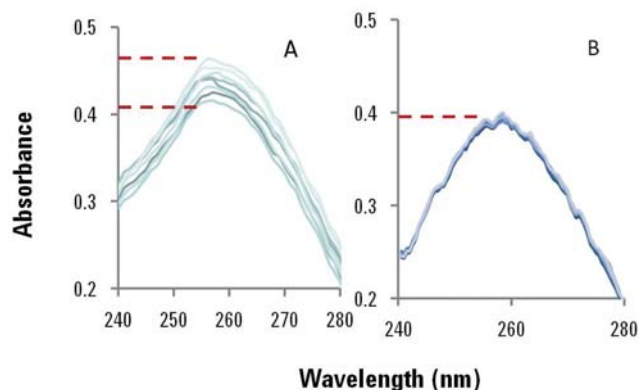


Figure 3. Eight repeated wavelength scans of 21.7 ng/ μ L DNA using a commercially available instrument (A), and the Cary 60 with ultra-microvolume cuvette (B) normalized for a 10 mm path length.

The absorbances being measured are the equivalent of 0.04 Abs at 260 nm with 1 mm path length, and with repeat measurements performed on the same sample. The exceptional instrument stability and repeatability can be seen. Clearly the Cary 60 instrument produces more reproducible wavelength scans for micro volume analysis where sample volumes are limited (Figure 3, plot B). The variability observed in Figure 3, plot A could result in errors of up to 15%.

Summary

Routine measurements of microvolumes take place in every bioscience laboratory. These include quantitative and qualitative measurements of nucleotides after purification or before molecular recombinant techniques such as amplification, ligations or transformations. For samples that are subject to degradation and are often at low concentrations, such as oligonucleotides and mRNA, the ability to quickly measure a small amount of sample using the Cary 60 with ultra-microvolume cuvette provides immense workflow advantages. As well as the analysis of nucleotides, proteins can also be measured to confirm purity and concentration. The analytical performance of the Cary 60 also allows users to extend their measurement capabilities through the use of accessories such as fiber optic dip probes or temperature controlled cuvettes.

Ordering information

Description	Part number
Cary 60 UV-Vis spectrophotometer	G6860A
Ultra-microvolume cuvette, 1 mm path length	G6871A

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