High throughput DNA quantification and quality checks for low volume samples

Using Tecan's Spark™ multimode microplate reader and patented NanoQuant Plate™ for DNA quantification, purity checks and full spectral analysis in small volumes

Introduction

Fast and reliable nucleic acid quantification and purity checks are becoming increasingly important for many laboratories, driven by the widespread use of next generation sequencing and other high throughput nucleic acid analysis techniques. Many methods require rapid, accurate analysis of small sample volumes to keep pace with the downstream processes.

The new Spark multimode microplate reader is equipped with patent-pending High Speed Monochromators (HSM) enabling highly accurate, reproducible and ultra-fast absorbance measurements. This unique optical system offers an enhanced measurement range from 200 to 1,000 nm, ensuring optimal performance across the full range, particularly for absorbance measurements in the deep UV range, such as A_{260}/A_{230} nucleic acid purity checks. The Spark reader is also suitable for measurements of low volume samples in both absorbance and fluorescence modes using the patented NanoQuant plate (Figure 1). This unique quartz optic allows researchers to measure up to 16 samples simultaneously, using just 2 µl sample volumes.

The most popular technique for determining nucleic acid concentration is based on measuring the absorbance at 260 nm (A_{260}). The purity of the DNA or RNA sample is can also be assessed by comparing absorbance values at 230, 260 and 280 nm (260/280 ratio and 260/230 ratio). A 260/280 ratio value of ≤1.8 for DNA or ≤2.0 for RNA indicates contamination of the sample with proteins (aromatic groups) and phenols, and a 260/230 ratio ≤2.0 indicates contamination with carbohydrates, salts or organic solvents.

Figure 1: Tecan's NanoQuant Plate for low volume nucleic acid quantification.

The SparkControl™ software offers a preconfigured, 'one-click' application for nucleic acid quantification, making it extremely quick and easy. The reader automatically measures absorbance at 230, 260 and 280 nm, as well as performing a fast spectral scan from 200 to 1000 nm (in 1 nm increments), typically taking just five seconds per sample and avoiding issues associated with sample evaporation. The resulting

absorbance spectrum can then be displayed and analyzed using the SparkControl software.

This technical note describes the use of the Spark reader for low volume DNA quantification, using the NanoQuant Plate to quantify 2 µl DNA samples – including a full spectral analysis – in just a few seconds.

Materials and methods

- Spark 10M multimode microplate reader
- NanoQuant Plate
- 96-well UV-Star®, flat bottom, transparent microplates (Greiner Bio-One, Austria)
- Tris-EDTA (TE) buffer (BioThema, Sweden)
- Phage Lambda-DNA, 300 µg/ml (Invitrogen, USA)
- 70 % ethanol
- ddH_2O

Measurement parameters and instrument settings

The SparkControl software enables easy selection of the “NanoQuant Nucleic Acid Quantitation” control bar. Two distinct blanking options are available; individual blanking (set by default) and average blanking. For individual blanking, blank values for each sample position are subtracted from the sample values measured in the same position. For average blanking, the sample measurement values are corrected against the average value of all positions used for the blanking procedure. Figure 2 illustrates the workflow panel of the SparkControl software which appears after blanking followed by the sample measurement.

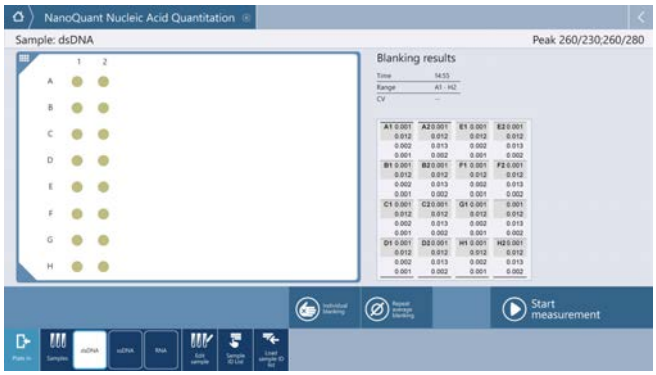

Figure 2: Application stripe after blanking.

All wavelengths for the nucleic acid quantification are measured automatically by the SparkControl software, using 310 nm as a reference wavelength for internal correction. The measurement results include a full spectrum from 200 to 1,000 nm, as well as the 260/280 and 260/230 ratios.

DNA quantification and purity checks

Prior to performing the measurements, the plate was cleaned using blank solution according to the Quick Guide for the NanoQuant Plate [1]. 16 replicates of four different concentrations (shown in Table 1) were measured.

Dilution	Concentration (µg/ml)
A	50
B	25
C	12.5
D	6.25

Table 1: DNA concentrations used for quantification and purity checks.

OD_{260} linearity

The measurement linearity at 260 nm was measured with a Phage Lambda-DNA dilution series. The Phage Lambda-DNA was serially diluted 1:3 in EDTA buffer, as summarized in Table 2. Eight replicates for each concentration were averaged and blanked, and the corrected average OD_{260} values were plotted.

Dilution	Concentration (µg/ml)
A	300
B	100
C	33.3
D	11.1
E	3.7
F	1.4

Table 2: Dilution series of Phage Lambda-DNA

Results

DNA quantification and purity checks

The average values after referencing and blanking are listed in Table 3, clearly demonstrating that the DNA concentrations calculated using the SparkControl software perfectly correlate with the theoretical concentration of the samples. 260/280 and 260/230 measurements are well within the expectation for pure DNA samples. The measurement uniformity (% CV across all 16 replicates) is good, ranging from 1.44 to 8.54 %. The theoretical detection limit for DNA measured in this application was calculated to be below 1 µg/ml.

DNA conc. (µg/ml)		% CV	260/280 ratio	260/230 ratio
Theoretical	**Measured**			
50	50.4	4.73	1.87	2.03
25	26	1.44	1.88	2.07
12.5	12.1	5.53	1.89	2.09
6.25	6.9	8.54	1.90	2.09

Table 3: Results of the DNA quantification and purity check

Figure 3 shows a DNA spectrum between 215 and 300 nm for a single DNA sample after blank reduction. The resulting spectrum is of high quality, providing additional information on the sample purity which can be used to increase the efficiency of analysis and help to optimize productivity in the lab.

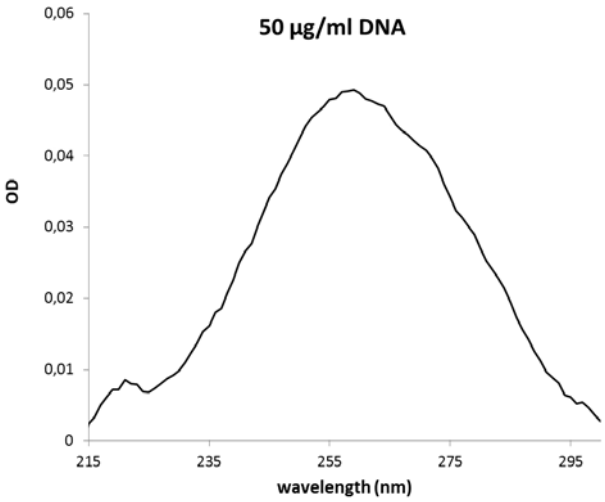

Figure 3: DNA spectrum (50 µg/ml sample) after blank reduction measured in the NanoQuant Plate using 2 µl sample volume.

OD_{260} linearity

Figure 4 shows the measurement linearity at 260 nm using the DNA concentrations outlined in Table 2. The R^2 of the dilution curve is typically above 0.999 between OD values of 0 and 3.5. This ensures a broad measurement range, avoiding the need for dilution of high concentration samples, further increasing the effectiveness and productivity of the assay.

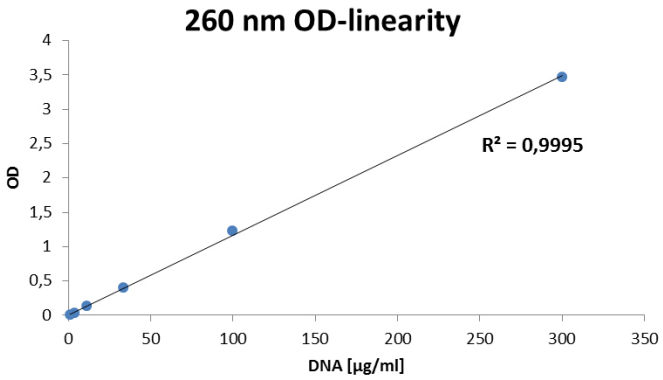

Figure 4: Measurement linearity at 260 nm.

Summary

Tecan's new Spark multimode microplate reader is equipped with High Speed Monochromators, offering ultra-fast scanning capabilities with full spectrum acquisition in under five seconds per sample and a high linearity for DNA quantification.
This ingenious hardware design is supported by a preconfigured 'one-click' software application for nucleic acid quantification and purity determination.

The results of this study show that combining the new Spark reader with the NanoQuant Plate provides a reliable, efficient tool for the quantification of nucleic acids in low volume samples. The dedicated software application combines nucleic acid quantification and purity checks (260/280 and 260/230 ratios) with a fast scan of the full spectrum from 200 to 1,000 nm, providing valuable additional data for further analysis.

References

(1) Quick Guide NanoQuant Plate™ No.30035094 Rev No. 1.4

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