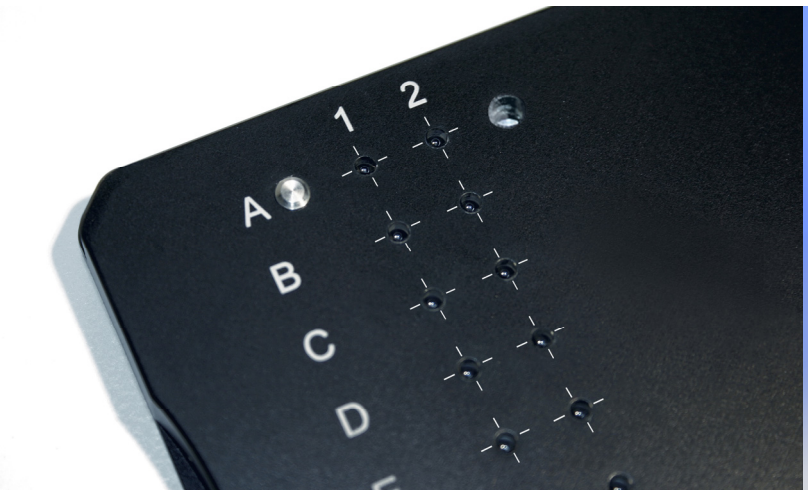
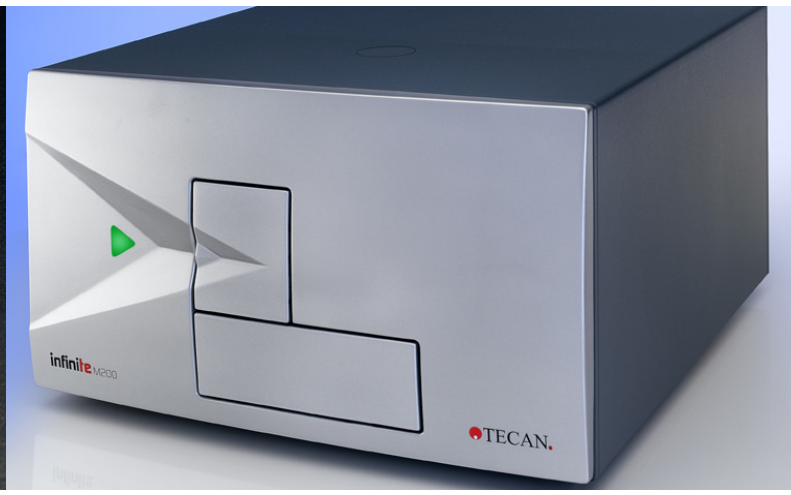
Low Volume DNA and RNA Quantification

NanoQuant Plate™ for use on Infinite® F200 and Infinite® M200





Introduction

Many recent genetic and forensic tests require the detection and quantification of very small amounts of nucleic acids. The most popular technique for determining nucleic acid concentrations is based on measuring the absorbance at 260 nm (A_{260}). The purity of the DNA or RNA is checked by comparing absorbance values from 260 nm and 280 nm measurements (260 / 280 ratio).

Real time PCR assays and array hybridization experiments require dye-labeled probes. One important parameter in achieving comparable results is the degree of labeling of the DNA or RNA probe with a certain dye (labeling efficiency).

The new NanoQuant Plate from Tecan allows researchers to measure the concentration and purity of nucleic acids of up to 16 samples simultaneously. Additionally, the efficiency of the nucleic acid labeling with dyes can be easily determined with this new tool.
For these applications only 2 µl of the sample volume is required, making the NanoQuant Plate an ideal tool for the latest applications in molecular biology.

Material and Methods

Instruments

- Infinite F200 filter-based detection system (Tecan Austria)
- Infinite M200 Quad4 Monochromator detection system (Tecan Austria)

NanoQuant Plate

- NanoQuant Plate with 16 (2 x 8) separate sample positions with specially designed quartz optics (Tecan Austria)

Figure 1: Tecan's NanoQuant Plate. The figure shows the NanoQuant Plate with its lid closed.

Each NanoQuant Plate is delivered with an optimized plate definition file (pdfx) for accurate positioning within the reader.

Reagents

- 20x TE (Invitrogen, CA)
- lambda-DNA, 100 µg/ml (Invitrogen, CA)
- Nuclease free water (Fluka BioChemika, Switzerland)

Phage lambda-DNA was diluted in 1x TE using nuclease free water to final concentrations of 5, 10, 20 and 50 µg/ml

Measurement Parameters and Settings

For DNA quantification and purity check measurement parameters for Infinite M200 and Infinite F200 instruments are shown below. As a reference wavelength for internal correction, the absorbance was set at 310 nm for the Infinite M200 and at 340 nm for the Infinite F200 instruments.

Measurement parameters for Infinite 200 instruments	
Parameter	**Setting**
Absorbance	260 nm
Bandwidth	5 nm
Absorbance	280 nm
Bandwidth	5 nm
Settle time	0 ms
Number of reads	25

Table 1: Measurement parameters for Infinite M200 and Infinite F200 with Tecan i-control™ software.

For easy handling in Tecan's i-control software, an application tab was introduced to allow the selection of the control bar "Quantify Nucleic Acid", among other items.
In Figure 2 the strip is shown as it appears in the workflow pane of the i-control software after blanking followed by the sample measurement.

Figure 2: Quantify Nucleic Acid Strip as it appears in the i-control Software after blanking.

Before the measurement was performed, the NanoQuant Plate was cleaned according to the Quick Guide for the NanoQuant Plate [1].
Samples were pipetted after blanking on the NanoQuant Plate quartz positions. For sample application a multi-channel pipette was used.

Results

Measurment results of blanking and referencing, nucleic acid quantification, as well as a purity check and the results for determining the labeling efficiency are displayed in Excel® automatically for all 16 measurment positions. In Figure 3 only the result of one well (A1) is shown for demonstration.

DNA – Quantification and Purity Check

DNA samples ranging from 5 to 50 µg/ml were measured using the NanoQuant Plate. The average values after referencing and blanking are listed in Table 2.

DNA [µg/ml]	5	10	20	50
Abs 260 [OD]	0.0047	0.0082	0.0184	0.0489
Abs 280 [OD]	0.0023	0.0042	0.0098	0.0259
Ratio 260/280	2.07	1.95	1.88	1.88

Table 2: Absorbance measurements of DNA samples from 5 µg/ml to 50 µg/ml. Results are blank corrected using the NanoQuant Plate.

The detection limit for nucleic acid quantification using the NanoQuant Plate was calculated to be about 1 µg/ml (or 1 ng/µl).

Labeling Efficiency

An amplified cDNA probe was labeled with Cy5 and measured using the NanoQuant Plate.

SampleType: dsDNA
Cy5: 649 nm

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		Abs		Value	
A	260	0.2452	OD	**191.20**	**ng/µl**
	280	0.1563	OD	**1.75**	**ratio**
	Cy5	0.2093	OD	**15.61**	**pmol/µl**
	None		OD		**pmol/µl**

Figure 3: Excel sheet output of the measuring results for position A1. The raw data for 260 nm, 280 nm and Cy5 absorbance are shown in the left columns. Calculations to get the values for nucleic acid concentration, purity check and dye concentration are shown in the right columns.

For additional information about the Cy5 labeled probe, the Infinite M200 scanning option was used to generate an absorbance scan from 230 – 700 nm (see Fig. 4)

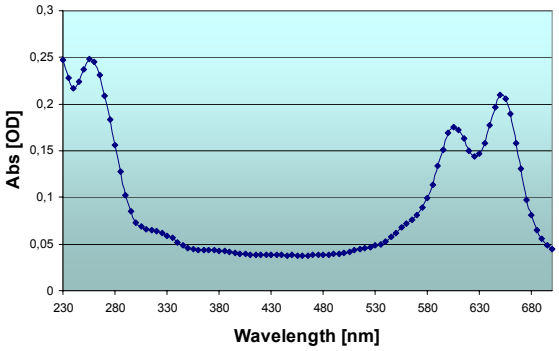

Figure 4: Absorbance scan of Cy5 labeled cDNA probe. Scan from 230 – 700 nm, bandwidth 5 nm, raw values are plotted (non referenced, non-blanked) for generating the curve. Note that Cy5 has two absorbance peaks (605 and 649 nm).

Conclusion

In many modern molecular biological applications sample size has become increasingly smaller and the determination of nucleic acid concentrations from these small volumes has become an increasingly demanding question in modern molecular biology. In forensic analysis DNA probes for genetic fingerprinting are sometimes taken only from a small number of cells.
With its NanoQuant Plate, Tecan provides a new tool for reproducible and sensitive quantification as well as purity check of nucleic acids.
For quality control of oligonucleotide labeling reactions in real-time PCR assays and in array hybridization experiments using dye-labeled probes, the labeling efficiency is an important parameter to evaluate results. The NanoQuant Plate can be easily used to determine the labeling efficiency of nucleic acid probes.
The unique mathematic algorithm, which references every measurement position used for calculations, makes the NanoQuant Plate an ideal tool for getting reliable and accurate results.

Literature

[1] Quick Guide NanoQuant Plate™ , 2008

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