



Low Volume DNA Quantification for Affymetrix® GeneChip

NanoQuant Plate™ for use on Infinite® 200 NanoQuant



Introduction

Scientists from Affymetrix® Inc., a world-leading supplier of microarray equipment and assays, evaluated the Tecan Infinite® 200 NanoQuant multimode microplate reader and two other spectrophotometers for DNA concentration analysis.

Detection, quantification and the assessment of purity of biomolecules in very small volumes are the basis of many modern assay systems.

The most used traditional method determining nucleic acid concentrations is based on measuring the absorbance at 260 nm (A_{260}). The purity of DNA or RNA is checked by comparing absorbance values from 260 and 280 nm measurements (260 / 280 ratio) and it is generally accepted that a sample of pure, double-stranded DNA (dsDNA) of 50 µg/ml has an absorbance at 260 nm of 1.0 and the ratio 260/280 nm will be greater than 1.8 [1].

The NanoQuant Plate™ from Tecan allows researchers to measure the concentration and purity of nucleic acids from up to 16 samples simultaneously.

Additionally, the efficiency of nucleic acid labeling with fluorescent dyes can be easily determined with this new tool. For these applications only 2 µl from the sample volume is needed, which makes the NanoQuant Plate an ideal tool for the latest applications in molecular biology.

In this note, data are presented from a comparison done by Affymetrix using Tecan's Infinite 200 NanoQuant, Molecular Devices SpectraMax® Plus and the Nanodrop® ND1000 instrument.

Material and Methods

NanoQuant Plate

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



Figure 1: Tecan's NanoQuant Plate. The figure shows the NanoQuant Plate with closed lid as it will be read by the Infinite 200 readers, see also [2].

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

Instruments

- Infinite M200 NanoQuant Quad4 Monochromator detection system (Tecan Austria)
- SpectraMax Plus, Molecular Devices USA
- Nanodrop ND 1000, Thermo Scientific, NanoDrop Products, USA

Reagents

- Human genomic DNA was selected to use to quantify the concentration. DNA sample preparation and DNA quantification before and after whole genome amplification were done at Affymetrix.
- A Promega Human genomic DNA was selected to use to quantify the concentration.

Measurement Parameters and Settings

For DNA quantification the measurement parameters and instrument settings for Infinite 200 NanoQuant are shown below.

| Measurements on 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 and settings for the Infinite 200 NanoQuant using Tecan’s i-control™ software.

For easy handling with Tecan’s i-control an application tab was introduced to select for example. ‘Quantify Nucleic Acid’ in the control bar. 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.

For measuring with MD’s SpectraMax Plus a 100-fold dilution has to be prepared and 200 µl pipetted in a 96-well UV-transparent microtiter plate before DNA quantification.

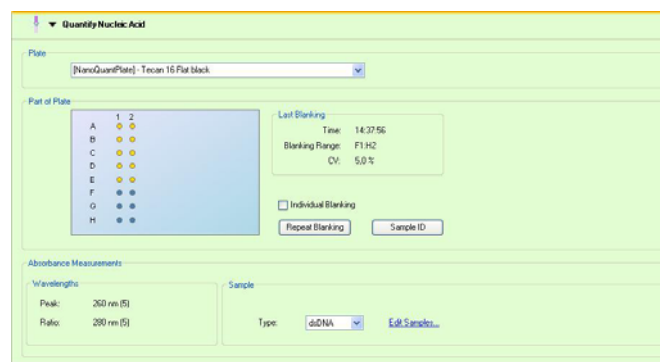


Figure 2: Quantify Nucleic Acid strip as it appears in the i-control software after blanking.

Samples were pipetted after blanking onto the NanoQuant Plate quartz positions. For sample application a multi channel pipette was used.

Two independent experiments were performed. In the first experiment genomic DNA from different origins and concentrations were used. In the second experiment a Promega Human genomic DNA was diluted to 1:1 dilution and 1:10 dilution. All samples were measured 3 to 6 independent times for each on Tecan Infinite 200 and the same DNA samples were then measured on the other two instruments in the lab ND1000 and SpectraMax Plus.

| Sample | Tecan NanoQuant | | MD SpectraMax Plus | | NanoDrop ND1000 | |
|--------------------------|-----------------|----------|--------------------|----------|-----------------|------------|
| | Average | CV [%] | Average | CV [%] | Average | CV [%] |
| Purified ProMale (n=6) | 1080.3 | 1 | 1141.2 | 4 | 1009.4 | 0.8 |
| Purified ProFemale (n=6) | 999.8 | 0 | 1073 | 2 | 946.3 | 0.7 |
| NA 19219 (n=6) | 869.5 | 1 | 893.2 | 3 | 815.4 | 1.6 |
| NA 19193 (n=6) | 1107.5 | 1 | 893.2 | 4 | 1069.6 | 1.7 |
| Average CV [%] | | 1 | | 3 | | 1.2 |

Table 2: Absorbance measurements of DNA samples using Tecan’s NanoQuant, MD’s SpectraMax Plus and the NanoDrop ND1000 instrument.

| Sample | Tecan NanoQuant | | MD SpectraMax Plus | | NanoDrop ND1000 | |
|--------------------------|-----------------|----------|--------------------|----------|-----------------|----------|
| | Average | CV [%] | Average | CV [%] | Average | CV [%] |
| ProMale (n=3) | 40.3 | 5 | 37.0 | 3 | 39.9 | 2 |
| ProFemale (n=3) | 52.8 | 3 | 44.3 | 9 | N/A | - |
| HapMapMale (n=3) | 46.0 | 1 | 38.3 | 8 | 46.3 | 4 |
| HapMapFemale (n=3) | 46.3 | 2 | 36.7 | 3 | 46.0 | 2 |
| Purified ProMale (n=3) | 1054.3 | 1 | 1001.3 | 1 | 1110.6 | 5 |
| Purified ProFemale (n=3) | 974.8 | 1 | 964.0 | 0 | 995.0 | 1 |
| Average [%] | | 2 | | 4 | | 3 |

Table 3: Absorbance measurements of PCR amplified DNA samples using Tecan’s NanoQuant, MD’s SpectraMax Plus and the NanoDrop ND1000 instrument.

Results

Measurement results for blanking and referencing, of nucleic acid quantification, purity check and results for determination of the labeling efficiency are displayed in Excel® automatically for all 16 measuring positions.

The results from experiment 1 are displayed in table 2 and 3 and the data derived from experiment 2 are shown in table 4.

The yields of the measurements are comparable between the three instruments, but the Tecan Infinite 200 NanoQuant exhibits an exceptionally high reproducibility (<3%).

DNA – Quantification

DNA samples ranging from about 40 to over 1000 ng/µl were measured using the NanoQuant Plate and for comparison with a MD’s SpectraMax Plus and the NanoDrop ND1000 instrument.

| Sample (n=3) | Tecan NanoQuant | | | MD SpectraMax Plus | | | NanoDrop ND1000 | | |
|--------------------------------|-----------------|-------|--------|--------------------|-------|--------|-----------------|-------|--------|
| | ng/µl | ratio | CV [%] | ng/µl | ratio | CV [%] | ng/µl | ratio | CV [%] |
| PromegaFemaleDNA | 227.6 | 1.8 | 1.0 | 217.9 | 1.93 | 4 | 215.7 | 1.86 | 2.2 |
| PromegaFemaleDNA 1:1 dilution | 112.6 | 1.8 | 0.1 | 118.7 | 1.98 | 2 | 106.9 | 1.88 | 0.7 |
| PromegaFemaleDNA 1:10 dilution | 22.1 | 1.8 | 2.3 | 25.3 | 1.96 | 5 | 20.6 | 1.99 | 3.7 |

Table 4: Experiment 2: Absorbance measurements of DNA samples using Tecan’s NanoQuant, MD’s SpectraMax Plus and the NanoDrop ND1000 instrument.

Conclusion

In many modern molecular biology applications sample size has become increasingly smaller and the determination of nucleic acid concentrations in these small volumes has become an even more demanding question in modern molecular biology. In forensic analysis DNA probes for genetic fingerprinting are sometimes only from a small number of cells.

With the NanoQuant Plate, Tecan provides a new tool for reproducible and sensitive quantification and purity check of nucleic acids.

The unique mathematic algorithm referencing every measurement position used for calculations makes the NanoQuant Plate an ideal tool to get reliable and highly accurate results.

For nucleic acid quantification and purity check, the Infinite 200 NanoQuant system is now listed by Affymetrix as a recommended instrument.

Literature

- [1] Maniatis T, Fritsch EF, Sambrook J (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Springs Harbor, NY
- [2] Quick Guide NanoQuant Plate™, 2008

User Statement

Vicky Huynh from the Product Development Organization at Affymetrix states:

“The Infinite M200 NanoQuant is one of my favorite quantification instruments in the lab. The software has many nice user-friendly interfaces. The data is automatically calculated and displayed in an Excel sheet after measurement. The measurement is quick and allows the measurement of 16 samples at a time in a minute or so. Also, there is no need to dilute samples, which saves time, diluting errors and plastic ware. Thus, to measure a plate, it would take less than 15 minutes. The instrument shows high reproducibility data <5%. With all these reasons, I would highly recommend the Infinite M200 NanoQuant as a quantification instrument. “

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