Application Note

MEDIUM-THROUGHPUT MICROVOLUME DNA QUANTIFICATION ON BERTHOLD TRISTAR MULTIMODE READERS

USING THE µDROP™ PLATE TO INCREASE LAB PRODUCTIVITY OF NUCLEIC ACID QUANTIFICATION

Abstract

Absorbance measurements of DNA, RNA and proteins are often performed using a microvolume spectrophotometer. However, this method can normally only be used to measure single samples, limiting the productivity of laboratories that need to analyse large numbers of samples. While microplate readers offer much higher throughput, standard microplates require large sample volumes that are often not available. The µDrop™ plate offers a solution to this problem. With 16 sample wells and a sample volume of just 2 µL, throughput is significantly increased compared to a microvolume spectrophotometer. Combined with a plate reader such as the Tristar multimode readers, it provides a medium throughput solution for nucleic acid quantification in combination with small sample volumes.

Introduction

DNA quantification is an important pre-analytical method, which is of great importance for many molecular biology analytical methods and can even determine their success. It is also a routine technique in procedures for translational research such as Next-Generation Sequencing (NGS), Polymerase Chain Reaction (PCR) or Real-Time PCR (quantitative PCR; qPCR), cloning or transfection, which initiates the subsequent workflow.

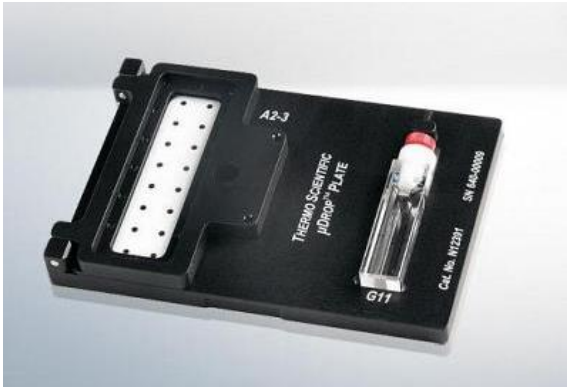

Figure 1: The µDrop™ plate can be used for DNA quantification using absorbance at 260 nm in a microplate reader using only 2 µL of sample. It also features a cuvette port for increased flexibility.

The most popular DNA quantification methods are based on UV-Vis- or fluorescence spectroscopy. Absorbance at 260 nm has been the method of choice for routine quantification of DNA and RNA since decades. It is simple and convenient to use as no

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further sample treatment (other than DNA extraction) is required. However, it is not very specific (it measures all nucleic acids as a whole) and it is sensitive to contaminants, so it demands very pure DNA to be accurate. Many of those contaminants can be estimated by measuring the absorbance of the sample at wavelengths other than 260 nm (usually at 230, 280 and 340 nm).

Absorbance of DNA samples at 260 nm is currently measured most often using a microvolume spectrophotometer (such as the Colibri+ from Berthold), but it is also possible to use a microplate reader. Microplate readers can measure many samples in a short time (typical plate formats are 96- and 384-well), but they require larger sample volumes for the measurement than a microvolume spectrophotometer (up to 50 µL in standard 96-well plates, less in other microplate formats). However, there is also the µDrop™ plate. It uses small sample volumes (from 2 µL). While it doesn't have as many sample positions as standard microplates (16 instead of 96), it offers a good compromise between sample volume and throughput. It also features a cuvette port, which allows to measure cuvettes in the microplate reader.

In this Application Note we demonstrate the suitability of the Tristar Multimode Microplate Readers or the quantification of dsDNA using the µDrop™ plate.

Materials

- Tristar 5 Multimode Microplate Reader, Berthold Technologies (Id. Nr. 69185-15).
- Tristar 3 Multimode Microplate Reader, Berthold Technologies (Id. Nr. 69173-30).
- µDrop™ microvolume plate (Id. Nr. 64154).
- Invitrogen™ Salmon Sperm DNA, sheared (10 mg/mL), Thermo Fisher (Cat. #AM9680).
- Nuclease-free water.
- Tubes of various volumes.
- Pipettes and pipette tips (various volumes).

Methods

The DNA stock was diluted to a concentration of 1600 ng/µL using TE buffer. A ½ dilution series was prepared using this diluted stock, producing the following DNA concentrations: 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/µL DNA.

To perform the blank measurement, 2 µL TE buffer were pipetted in duplicate in each spot of the µDrop™ plate and absorbance was measured at 260 and 340 nm. After removing the TE buffer from the plate, 2 µL of each DNA concentration were pipetted in the µDrop plate and absorbance was measured at the same wavelengths.

The plate was measured with the settings detailed under "Instrument settings". The absorbance at 340 nm was used for baseline correction, and blank values were subtracted from the absorbance values of the DNA solutions at the corresponding position. Data were exported to xls format, and standard curves were drawn in Excel.

In addition, the spectrum of the 1600 ng/µL standard was measured using a High-resolution scan (see "Instrument settings").

Tristar Series

Application flexibility you can count on

Developed for high flexibility and equipped with the proprietary ONE-4-ALL optical system, the Tristar Serie combines the user friendliness of a multimodal optical system with the sensitivity and performance of a dedicated optical device. You can choose between the affordable Tristar 3 and the more advanced and flexible Tristar 5. The Tristar series provides you with flexibility for today, tomorrow, and beyond.

- Monochromator Technology
- High-sensitivity Luminescence
- BRET
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- UV/VIS Fluorescence
- UV/VIS FRET
- Time-Resolved Fluorescence (TRF)
- Time-Resolved FRET (TR-FRET / HTRF®)
- Fluorescence Polarization
- AlphaScreen®
- Top and Bottom Reading
- Incubation

Instrument settings

Tristar 3:

- Reading mode: Absorbance
- Counting time: 0.10 s
- Use: Filters
- Aperture: 3 – Rd2
- Measurement Filters: 240/10, 260/10, 280/5, 340/26.

Tristar 5 (DNA concentration):

- Reading mode: Absorbance
- Counting time: 0.10 s
- Use: Monochromator
- Beam size: Narrow
- Aperture: Default
- Meas. Wavelength: 230, 260 280 and 340 nm
- Meas. Slit Width: 5 nm

Tristar 5 (spectrum):

- Reading mode: Absorbance Spectral scan
- Counting time: 0.10 s
- Scanning quality: High resolution (slow)
- Beam size: Narrow
- Aperture: 3 - Rd 2
- Meas. Start Wavelength: 230 nm
- Meas. End Wavelength: 340 nm
- Increment Wavelength: 1 nm
- Meas. Slit Width: 5 nm

For the best performance we recommend creating a new plate profile for the µDrop™ plate using the Plate Editor and the following parameters:

- Number of rows: 8
- Number of columns: 12
- Height of plate: 14.40 mm
- Stacking height: 13.50 mm
- Distance from corner and from well to well: 15.10 mm (top), 11.60 (left), 9.00 (right), 9.0 (bottom)

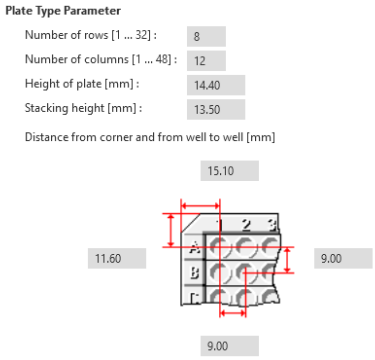

Figure 2: parameters for the µDrop™ plate as seen in the Plate Editor.

Results

The absorbance spectrum measured with the Tristar 5 was clean and with the expected shape for a high-purity DNA (figure 3).

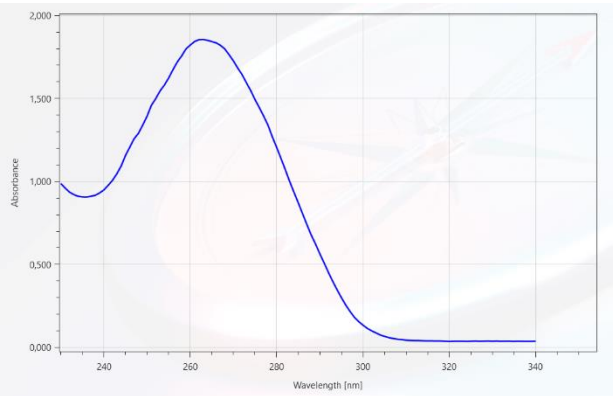

Figure 3: absorbance spectrum of the 1600 ng/µL DNA solution, measured at high resolution (1 nm scan).

The DNA standard curves obtained are displayed in figure 4. Linearity is excellent with both the Tristar 5 and the Tristar 3, with R^2 values of 0.9996 and 0.9993, respectively.

In preliminary measurements, the concentrations in positions pipetted first were overestimated (data not shown), probably due to evaporation during the time spent pipetting subsequent sample positions, as sample volume is small (2 µL) and the samples are exposed to air until the last sample is pipetted and the plate is closed. Hence, we recommend using a multichannel pipette to pipet blanks and samples in the µDrop™ plate.

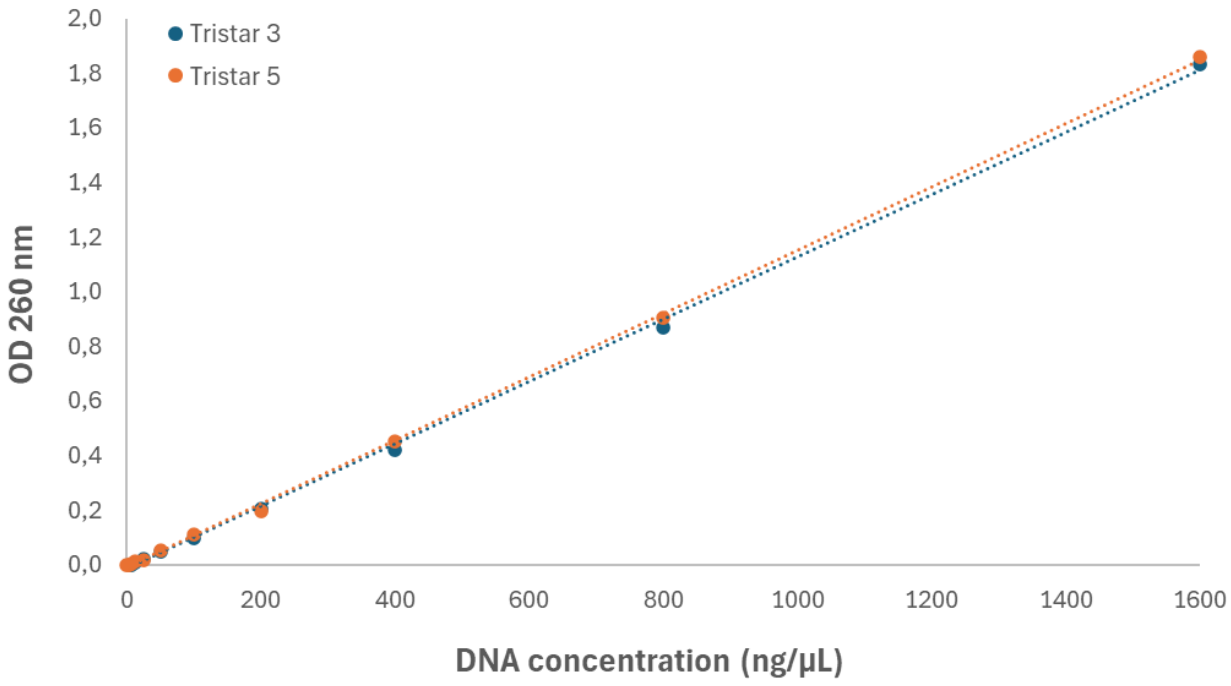

Figure 4: Dilution series of the 1600 ng/µL DNA solution, down to 1.563 ng/µL. Each standard was measured in duplicate in the µDrop™ plate.

Discussion and conclusions

Quantification of nucleic acids is very often performed using a microvolume spectrophotometer. However, this method suffers from low throughput, as samples have to be measured one by one. The µDrop™ plate, with 16 sample positions and a minimum sample volume of 2 µL, offers a higher throughput and, as displayed in the graphs above, can be used in both the Tristar 5 and Tristar 3 microplate readers. While it is possible to pipet the drops using a single-channel pipette, using a multi-channel pipette increases throughput and, most importantly, prevents inaccuracies due to evaporation.

All in all, the combination of the µDrop™ plate with the Tristar microplate readers is a useful tool for the quantification of nucleic acids for a range of applications, such as PCR, cloning, sequencing and others. It offers the means to simplify the workflow, while saving sample and improving the accuracy of the measurement.

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