Technical Note:
AN-MRµDrop_0911

DNA quantification in micro-liter volumes with Thermo Scientific µDrop Plate

SP&A Application Laboratory, Thermo Fisher Scientific, Vantaa, Finland

Key Words:
- Low volume
- Nucleic acid quantification
- DNA
- RNA
- 260/280
- LOD
- LOQ

This paper describes how to perform photometric nucleic acid quantification in small volumes with the Thermo Scientific µDrop Plate.
This note also describes the different performance parameters and instrument- dependent features affecting the performance of photometric measurements.

Introduction

UV photometry is a common way to quantify nucleic acids in a sample. Both DNA and RNA absorb UV light very efficiently, making it possible to detect and quantify the concentrations. Typical applications for this include, for example, the quantification of template prior to sequencing or PCR.
dsDNA is used as an example in this paper.

The photometric method is based on Lambert-Beer's equation and it utilizes the fact that the nitrogenous bases in nucleotides have an absorption maximum at about 260 nm.
The average extinction coefficient for double-stranded DNA is 0.020 $(\mu g/ml)^{-1}$ cm^{-1}. This means that 1.0 Abs at 260 nm corresponds to a concentration of 50 µg/ml for double-stranded DNA. Thus the amount of DNA can be calculated by using the formula:

DNA concentration (µg/ml) = Abs_{260} x 50 µg/ml

Unlike the nucleic acids, proteins have a UV absorption maximum at 280 nm, mostly due to the tryptophan residues. Therefore, the Abs_{260}/Abs_{280} ratio gives an estimate of the protein contamination of the sample. For a good quality sample, the value should be between 1.8 and 2.0. A value smaller than 1.8 indicates the presence of proteins and a value higher than 2.0 indicates probable contamination, such as phenols.

Another common parameter used to describe the quality of DNA is the 260-to-230 nm ratio. It is used to estimate chemical contamination, such as phenols, carbohydrates or a high salt concentration. The ideal 260/230 nm ratio is around 2.

A possible background caused by impurities in the sample can be corrected by a measurement made at a wavelength, at which the absorption level for nucleic acids and proteins is really low. The wavelength most commonly used for this background subtraction is 320 nm; (Abs_{260}-Abs_{320} / Abs_{280}-Abs_{320}). For example, magnetic beads are now commonly used in the nucleic acid purification process, and when bead-based purification is used, the 320 nm subtraction is always recommended.

The amount of sample available for the analysis is quite often really low, thus there is a need for a tool such as the µDrop™ Plate that enables these measurements at a microliter scale.
The µDrop Plate consists of two separate measurement locations, one for measuring low-sample volumes and the other for cuvettes (Figure 1).

Figure 1. µDrop Plate. A low sample volume measurement area with 16 measurement locations on the left and a cuvette location for a 10 mm cuvette on the right.

The low-volume measurement area consists of two quartz slides, the top clear quartz slide and the bottom partially Teflon-coated quartz slide. The bottom slide contains 16 sample positions, arranged in a 2 x 8 matrix, onto which samples can be pipetted. The cuvette slot of the plate is used to perform photometric measurements with standard cuvettes.

Compared to a normal cuvette; the pathlength of the µDrop Plate low-volume area is really short; 10 mm vs. 0.5 mm.
By decreasing the pathlength the sample volume can also be decreased. A sample volume down to 2 µl can be used with the µDrop Plate.
A shorter pathlength also increases the requirements of the photometer used as it reduces the measured absorbance. It is possible to measure DNA concentrations from a few nanograms to thousands of nanograms per microliter with a µDrop Plate and a photometer with high precision and a wide linear range.

Due to the optical light path of the µDrop Plate (0.5 mm), a multiplication factor of 20 must be taken into account in the concentration calculations, whereby

DNA concentration (µg/ml) = Abs_{260} x 50 µg/ml x (10 mm/0.5 mm) = Abs_{260} x 50 x 20

Any photometric measurement device, cuvette, microplate or µDrop Plate, always has a certain background absorption. Therefore, blank subtraction is always necessary when photometric quantification of the sample concentrations is performed.

Material and Methods

- Thermo Scientific µDrop Plate, N12391
- Thermo Scientific Multiskan GO microplate and cuvette spectrophotometer, Thermo Scientific, 51119300
- Thermo Scientific Varioskan Flash multimode reader, Thermo Scientific, 5250030
- Semi-micro cuvette, Hellma, 114-10-40-QS
- Herring Sperm DNA, Promega, D1816
- TE buffer, 10 mM TRIS-HCl, 1 mM EDTA, pH 7.5

The DNA stock solution of Herring sperm DNA was serially diluted in a ratio of 1:2 into TE buffer to provide 7 sample concentrations.
The concentrations of the samples were measured with the µDrop Plate low-volume area, the cuvette position and the Multiskan GO cuvette port. The concentrations measured with the Multiskan GO cuvette port were used as references in all the calculations.

The sample volume used was 4 µl in all low-volume area tests.
For the cuvette measurements, the DNA samples were diluted 1:20 to keep the absorbance values within the linear measurement range also with regard to the 10 mm light path.

a) Thermo Scientific SkanIt Software measurement protocols

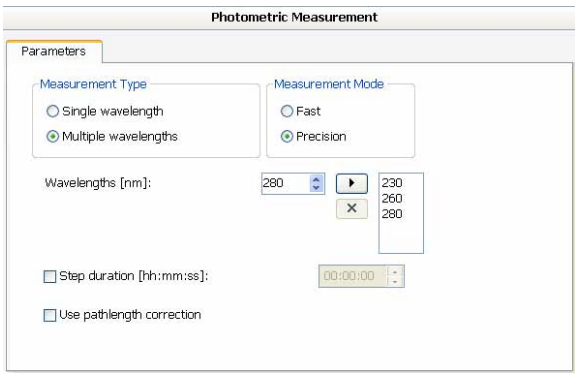

Figure 2. Measurement parameter setup of SkanIt for Multiskan GO

The following measurement steps were used in a DNA endpoint measurement (Figure 2): Photometric measurement, Measurement type: Multiple wavelengths, Measurement mode: Precision, Wavelengths: 260, 280, and 320 for both Multiskan® GO and Varioskan® Flash.

The concentration and Abs_{260}/Abs_{280} ratio can be calculated by the Precalculation or User Defined Equation steps in SkanIt® Software.
A quality certificate, providing the verified pathlength of each plate, is delivered with the µDrop Plate. This exact pathlength value should always be used for the concentration calculations instead of the nominal value of 0.5 mm to obtain more accurate results.

A blank measurement was made with 16 replicates before the concentration measurements. The average of the blank samples was subtracted from all unknown absorbances.

b) Terminology

The sensitivity of the assay is determined according to IUPAC with two different parameters: Limit of Detection (LOD) and Limit of Quantification (LOQ)[1]. LOD means the lowest amount of analyte that can be separated from the background. It is calculated based on the calibration curve slope vs. the blank + 3 x SD of the blank. LOD means, that this amount of analyte can be detected with statistical significance, but not necessarily quantified as an exact value.

LOQ is the lowest amount of the analyte at which quantification is possible with statistical relevance. It is defined as the quantitative detection limit as C_{ld} = ks/m, where k is 10, s is the standard deviation of instrument readings taken on blank, and m is the slope of a plot of instrument response vs. concentration, as calculated by linear regression. In practice, LOD is the limiting value in qualitative assays where a simple yes/no answer to the question "Is there any analyte in my sample" is required and LOQ is the limiting value that the user can measure as the concentration of the analyte in quantitative assays.

c) µDrop detection range

With a µDrop Plate type of a measurement device, the maximum measurement range is always determined by the instrument. The lower part is determined by the precision of the blank (LOD) and the upper part by the linear range of the instrument. Thus both the theoretical minimum and maximum concentrations that can be measured on the

µDrop plate are instrument dependent and can be calculated from the given instrument specifications. For example, with an instrument that has a given precision specification of 0.001 Abs and a linearity up to 4 Abs, the maximum concentration range is from 3 x 0.001 x 50 µg/ml x 10 mm/0.5 mm)= **3 µg/ml** to 4 x 50 µg/ml x 20= **4000 µg/ml**.

Results

The results obtained with the µDrop Plate low-volume area and the cuvettes were compared to the results of the Multiskan GO cuvette port results.

A)Low-volume area

Sensitivity

The limit of detection and quantification were calculated as described above. An average value of several µDrop Plate measurements are shown in Table 1.

	Multiskan GO	Varioskan Flash
Limit of detection (LOD)	1.7 µg/ml	2.2 µg/ml
Limit of quantification (LOQ)	5.6 µg/ml	7.2 µg/ml

Table 1. LOD and LOQ values of the DNA quantification assay with the µDrop Plate and the Multiskan GO or Varioskan Flash

These values are well within the precision specifications of the Multiskan GO and Varioskan Flash, 9 µg/ml and 3 µg/ml, respectively. Based on this study, the Multiskan GO typically performs much better than specified.

An example of a dilution series measurement with the µDrop Plate with the Multiskan GO and Varioskan Flash is shown in Figure 3. The Multiskan GO cuvette port is used as the reference.

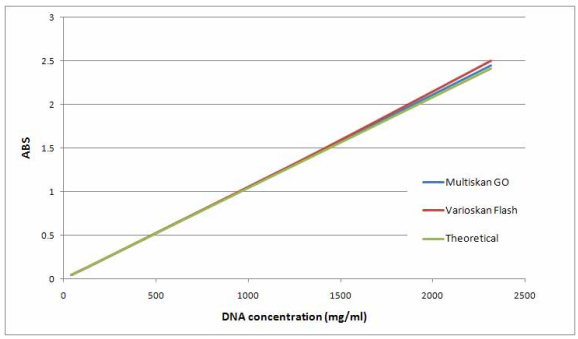

Figure 3. Example of a µDrop Plate DNA dilution series measurement

Detection range

The measured concentrations with the Drop Plate low-volume area with the Multiskan GO and Varioskan Flash were compared to the values of the Multiskan GO cuvette port (Figure 4 and Figure 5).

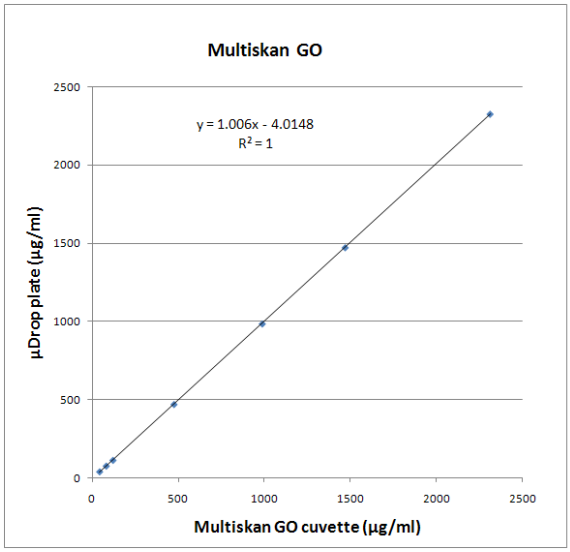

Figure 4. Correlation of the Multiskan GO µDrop Plate low-volume area to the Multiskan GO cuvette measurements

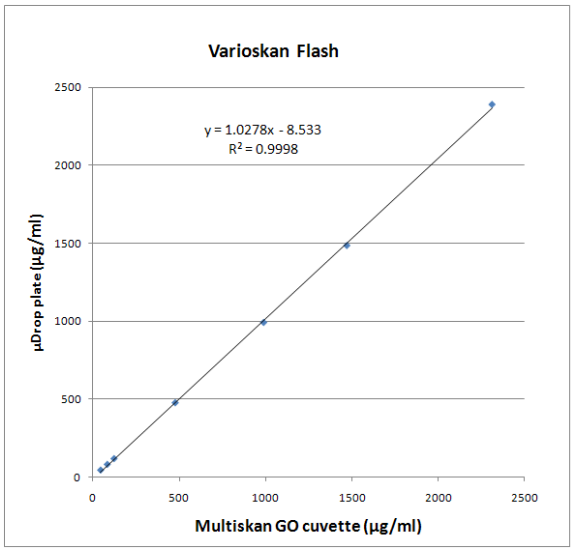

Figure 5. Correlation of the Varioskan Flash µDrop Plate low-volume area to the Multiskan GO cuvette measurements

Both systems showed excellent linearity up to the highest sample concentration: > 2000 µg/ml.

B)Cuvette measurement

The measured concentrations with the µDrop Plate cuvette adapter on the Multiskan GO were compared to the values of the Multiskan GO cuvette port (Figure 6).

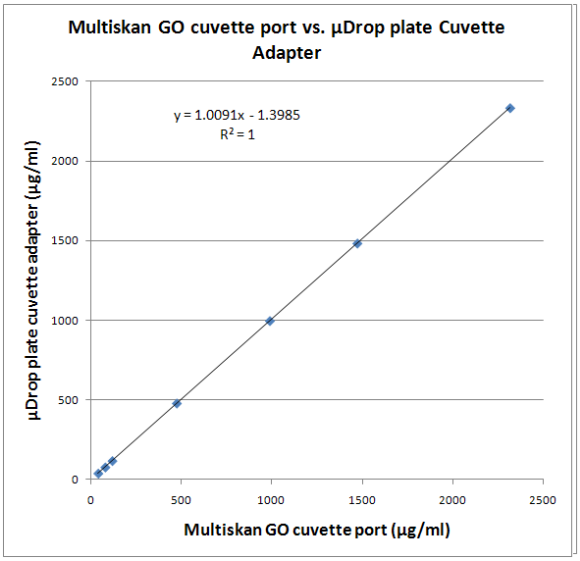

Figure 6. Correlation between the µDrop Plate cuvette adapter and the Multiskan GO cuvette measurements

The cuvette measurements of the µDrop Plate correlate perfectly with the cuvette port measurements.

Summary

- It is possible to measure nucleic acid concentrations from micrograms to even milligrams per milliliter with the µDrop Plate.
- The fixed pathlength of the plate enables direct calculation of the nucleic acid concentrations.
- The results of the low-volume area correlate very well with the cuvette measurement results.

References

1) Mocak et al., Pure and Applied Chemistry, Vol. 69 No. 2, pp 297–328, 1997.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America:
USA / Canada
+1 800 345 0206

Europe:
Austria
+43 1 801 40 0

Belgium
+32 53 73 42 41

France
+33 2 2803 2180

Germany national toll free
08001-536 376

Germany international
+49 6184 90 6940

Italy
+39 02 02 95059 448

Netherlands
+31 76 571 4440

Nordic countries
+358 9 329 100

Russia/CIS
+7 (495) 739 76 41

Spain/Portugal
+34 93 223 09 18

Switzerland
+41 44 454 12 12

UK/Ireland
+44 870 609 9203

Asia:
China
+86 21 6865 4588 or
+86 10 8419 3588

India toll free
1800 22 8374

India
+91 22 6716 2200

Japan
+81 45 453 9220

Other Asian countries
+852 2885 4613

Countries not listed:
+49 6184 90 6940 or
+33 2 2803 2180

www.thermoscientific.com/platereaders