Application Note:
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DNA quantitation in cuvette and TrayCell using the Thermo Scientific Multiskan GO spectrophotometer

Sample Preparation & Analysis Application Laboratory, Thermo Fisher Scientific, Vantaa, Finland

This application note describes how to perform photometric DNA quantitation and qualification measurements with the Thermo Scientific Multiskan GO microplate and cuvette spectrophotometer in various cuvette formats.

Key Words:
- DNA
- Photometric
- Quantitation
- TrayCell
- DNA Spectrum

Introduction

DNA quantification

Analysis of UV absorption is still probably the most common way to quantify DNA in a sample. The remarkable simplicity of the method compensates for its somewhat limited sensitivity. The method is based on the Lambert-Beer equation and utilizes the fact that nucleic acids have an absorption maximum at 260 nm.

Lambert-Beer equation: $A = \varepsilon bc$ where

ε = molar coefficient

b = pathlength

c = concentration

When the molar coefficient and pathlength are constant, absorbance is proportional to the concentration. For a standard cuvette spectrometer, the pathlength is usually 10 mm. In case the pathlength is something other than 10 mm, this needs to be taken into account.

The average extinction coefficient for double-stranded DNA is 0.020 $(\mu g/ml)^{-1}$ cm^{-1} at 260 nm. Therefore, 1 Abs at 260 nm corresponds to a concentration of 50 µg/ml for double-stranded DNA. The amount of DNA can be calculated by using the formula:

$$DNA\ concentration\ (\mu g/ml) = Abs_{260} \times 50\ \mu g/ml$$

Most samples contain contaminates such as proteins that maximally absorb at 280 nm. The ratio of Abs_{260}/Abs_{280} can therefore be used to analyze the purity of the sample. Values between 1.8 and 2.0 denote a good-quality sample. A ratio lower than 1.8 indicates the presence of proteins and a ratio higher than 2.0 indicates a probable contamination by, for example, phenols.

The possible background turbidity caused by impurities in the sample can be corrected by an absorption measurement made at wavelength of a low absorption level for DNA and protein. The wavelength most commonly used for this background subtraction is 320 nm (Abs_{260}-Abs_{320} / Abs_{280}-Abs_{320}).

Cuvette types

The cuvette type used for DNA measurements needs to be chosen on the basis of both material desired and sample available. The most common types and their approximate sample volumes are listed in Table 1.

Table 1. Different cuvette types and their typical sample volumes.

Cuvette type	Typical sample volume (µl)
Semi-micro	1000-3000
Ultra-micro	> 70
Ultra-micro measuring cell	0.7- 5.0

There are also two commonly used standards for spectrophotometer beam-heights (the height of the beam from the bottom of the cuvette): 8.5 mm and 15 mm. It is important to notice this when choosing the cuvette. For macro and semi-micro cuvettes, the height determines the minimum filling volume. For ultra-micro cuvettes there are two separate window height options available. The beam-height of Multiskan GO is 8.5 mm.

There are UV cuvettes of two types available: quartz and plastic. Plastic cuvettes are disposable, less expensive and have no possible sample carry-over. The quartz, however, has much better UV transmission and may be necessary where very high sensitivity is required. A third option for UV DNA/RNA quantitation is the use of ultra-micro measuring cells, such as Hellma TrayCell or Thermo Scientific NanoCell. Both have two optional optical light paths, 1 mm and 0.2 mm, which are chosen by changing the cell cap. The outer dimensions are equivalent to a standard cuvette.

With this type of device it is possible to reduce the sample volume to be used in the measurement, as the pathlength is much shorter than in traditional cuvettes. If the pathlength is reduced from 10 mm to less than a millimeter, the sample volume can be less than a microliter.

A shorter pathlength also makes high demands of the phototometer used, as it reduces the measured absorbance. A photometer with a wide dynamic range makes it possible to measure DNA concentrations from a few nanograms to thousands of nanograms/µl.

Due to the optical light paths of the TrayCell of 0.2 mm or 1 mm, a multiplication factor of 50 or 10 must be taken into account in the concentration calculations.

The Multiskan GO is a monochromator-based UV/VIS spectrophotometer. It is used in spectral scanning and endpoint and kinetic measurements to measure absorbance in the 200–1000 nm wavelength range from appropriate microplates and various types of cuvettes.

Multiskan GO has a pathlength correction option which automatically calculates the results for a 10 mm pathlength, despite the real pathlength used.

This note describes the quantification/qualification of DNA with different cuvette types for Multiskan GO.

Materials and methods

- Thermo Scientific Multiskan GO microplate and cuvette spectrophotometer (Thermo Scientific code 51119300)
- Cuvettes
  - Hellma 104-10-40-QS, Semi-Micro 1400 µl max volume, synthetic quartz
  - Helma 104.002F-10-40-QS, Micro Cell, synthetic quartz, pathlength 10 and 2 mm
  - Brand 759 150, UV-Transparent Disposable Cuvette, Semi-Micro, pathlength 10 mm
  - Brand 759 200, UV-Transparent Disposable Cuvette, Ultra-Micro, pathlength 10 mm
  - TrayCell, Hellma Analytics, 105.810-UVS, pathlength 0.2 and 1.0 mm
- Herring Sperm DNA, Promega D1816
- TE buffer, 10 mM TRIS-HCl, 1 mM EDTA, pH 7.5

A serial dilution series of Herring sperm DNA was made to TE buffer. The DNA stock solution was serially diluted to provide samples ranging from 5.1-10200 µg/ml. Different sets of the dilution were used with different cuvettes, depending on the cuvette pathlength. A spectral scan of the samples was also performed.

All cuvette types were measured using the identical procedure, but with different volumes and pathlengths (Table 2). The concentration range was chosen to be suitable for each cuvette type. Three replicates were made of each concentration, and each of these samples was measured with three separate Multiskan GO units.

Table 2. Cuvette types used.

	Cuvette type	Pathlength (mm)	Assay volume (µl)	Concentration range (µg/ml)
1	Hellma 104-10-40-QS, Semi-Micro	10	400	5.1 - 127.5
2	Hellma 105.810-UVS, TrayCell	0.2	1	255 - 5100
		1.0	3	25.5 - 1020
3	Hellma 104.002F-10-40-QS, Micro Cell	10	200	5.1 - 127.5
		2	200	17 - 680
4	Brand 759 150, Semi-Micro	10	400	5.1 - 127.5
5	Brand 759 200, Ultra-Micro	10	70	5.1 - 127.5

Both 0.2 and 1 mm pathlength options of the TrayCell and 10 and 2 mm pathlength options of the Hellma Micro Cell were tested and the pathlength correction was used to automatically make the results comparable.

The TrayCell was kept in the measurement position during the whole set of measurements. This guaranteed a continuously identical position of the aperture in the light beam and no variation in comparison to the blank measurement.

For Hellma Micro Cell, the pathlength change was made by rotating the cuvette 90°.

In Multiskan GO, the pathlength correction can be used to automatically normalize the measurement results to correspond to a 10 mm pathlength.

Measurement protocols

It is possible to control the Multiskan GO by two separate and alternative software programs. Thermo Scientific SkanIt software (b) allows the user to operate the system via PC, while internal software (a) enables keypad operation.

a) DNA quantitation with internal software

The instrument was first zeroed with empty cuvette chamber. Next, a blank cuvette with TE buffer is measured. Blank data was reported and if blank levels are acceptable, the reader is re-zeroed again with blank cuvette. The sample measurements were made using the ready-made dsDNA measurement mode of Multiskan GO:

Nucleotide: dsDNA
Pathlength correction: Yes

The pathlength was set according to the cuvette type. All DNA dilutions were measured using same procedure.

DNA spectra measurement of the dilutions were made with Spectrum mode:

Start wavelength (nm): 230 nm
End wavelength (nm): 350 nm
Step size (nm): 1 nm
Pathlength correction: Yes
Mode: Fast

Pathlength was set according to the cuvette type.

When a predefined DNA assay protocol is selected in the internal software, Multiskan GO automatically reports DNA concentration 260/280 both with and without 320 nm subtraction and the spectrum, in addition to measured absorbance values. The spectrum is also always displayed along with the measured and calculated results (Figure 1).

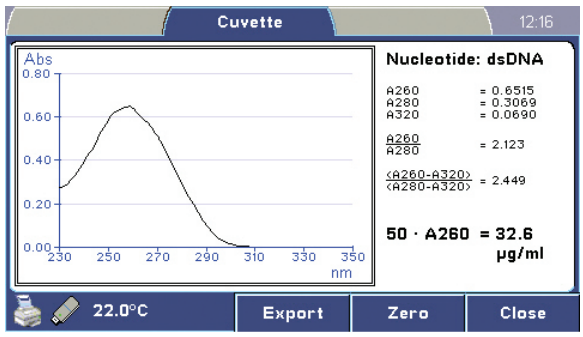

Figure 1. Example of Multiskan GO internal software results.

b) DNA quantitation with SkanIt software for Multiskan GO

A "layout" can be created for cuvettes in SkanIt software (Figure 2). Each cuvette can be classified, e.g., as a blank or control. This information then can be further utilized in calculations.

Figure 2. SkanIt layout for cuvettes.

When SkanIt software is used, the Multiskan GO is zeroed against the empty cuvette chamber and the buffer cuvette is always defined as blank in the cuvette layout. All cuvette types were measured using the identical procedure with different volumes and pathlengths. All DNA dilutions are measured using the same procedure; the same protocol was repeated for all three replicates.

The following measurement steps were used in DNA endpoint measurement:

Photometric measurement, Measurement type: Multiple wavelengths
Measurement mode: Precision
Wavelengths: 260, 280 and 320 nm

A spectral scanning was also made of each of the dilutions. The following measurement steps were used in DNA scanning measurement:

Photometric Spectrum Scanning, Scanning wavelengths:
Start (nm): 230 nm
End (nm): 350 nm
Step size (nm): 1 nm
Measurement Mode: Fast

The blank subtraction was calculated based on blank cuvette. Pathlength correction (PLC) was made for each measurement by SkanIt PLC calculation. The DNA concentration calculation was included into PLC (Figure 3).

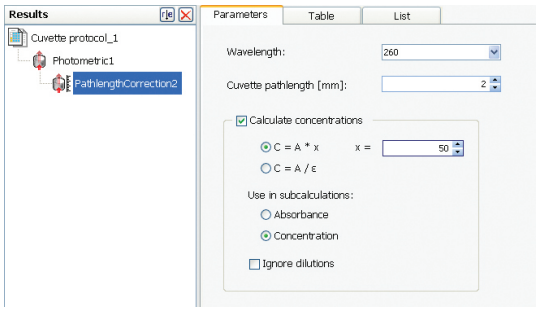

Figure 3. DNA concentration calculation in SkanIt software for Multiskan GO.

The Abs_{260}/Abs_{280} can be calculated by the precalculation step (Figure 4).

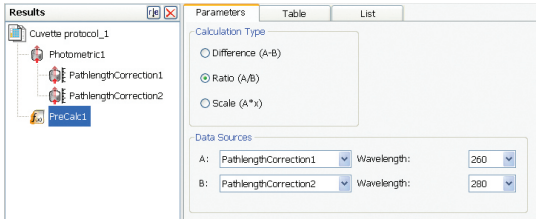

Figure 4. Abs_{260}/Abs_{280} calculation in Skanit software for Multiskan GO.

The measured concentrations were compared to the theoretical values calculated based on the values from the DNA reagent manufacturer.

Results and discussion

The results gained were compared to the theoretical values. The theoretical values were calculated from the concentration of the stock solution as reported by the manufacturer. Each result is an average of three measurements (n=3). It is possible to automatically calculate the DNA concentration with both SkanIt and internal softwares.

A graph of the calculated concentration was drawn for each cuvette type (red). In each graph the gained result can be compared to the expected graph (blue line) (Figures 5-11).

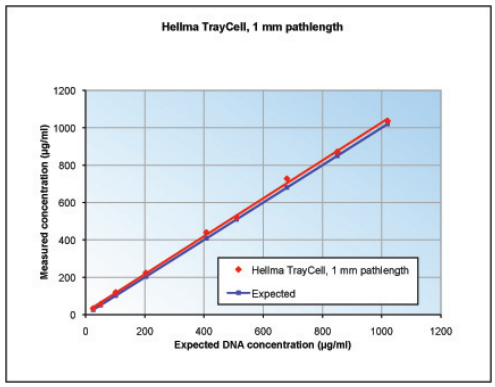

Figure 5. Concentration graph for Hellma TrayCell, 1 mm pathlength. Concentration range 25.5 - 1020 µg/ml.

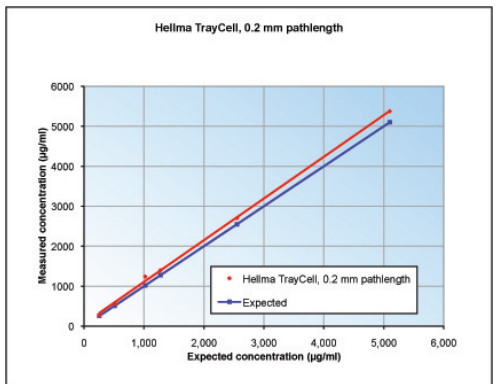

Figure 6. Concentration graph for Hellma TrayCell, 0.2 mm pathlength. Concentration range 255 -5100 µg/ml.

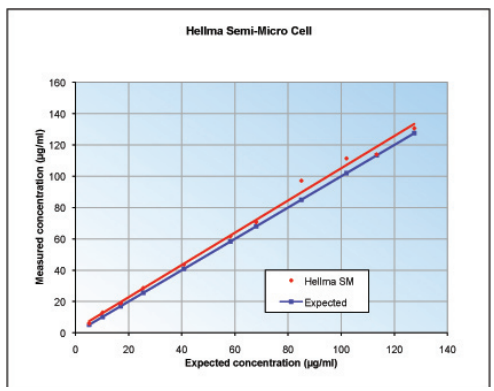

Figure 7. Concentration graph for Hellma Semi-Micro, 104-QS. Concentration range 5.1 – 127.5 µg/ml.

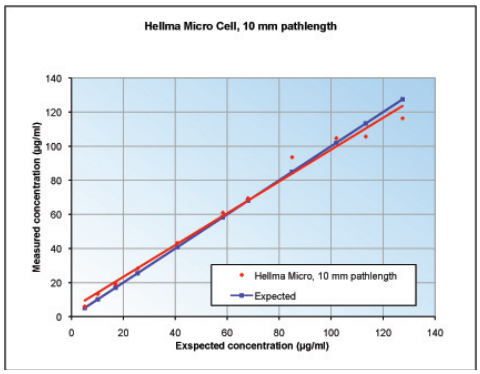

Figure 8. Concentration graph for Hellma Micro Cell, 104.002F-10-40-QS, 10 mm pathlength. Concentration range 5.1 – 127.5 µg/ml.

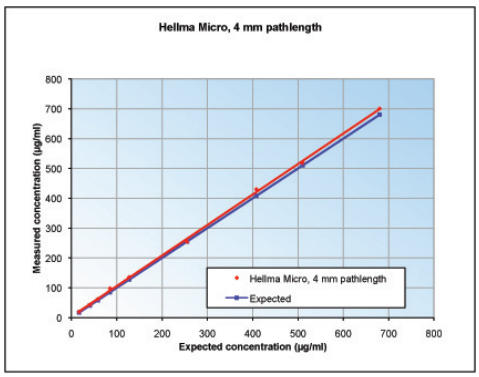

Figure 9. Concentration graph for Hellma Micro Cell, 104.002F-10-40-QS, 4 mm pathlength. Concentration range 17 – 680 µg/ml.

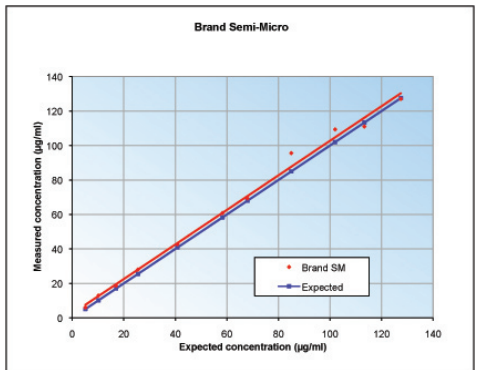

Figure 10. Concentration graph for Brand 759 150. Concentration range 5.1 – 127.5 µg/ml.

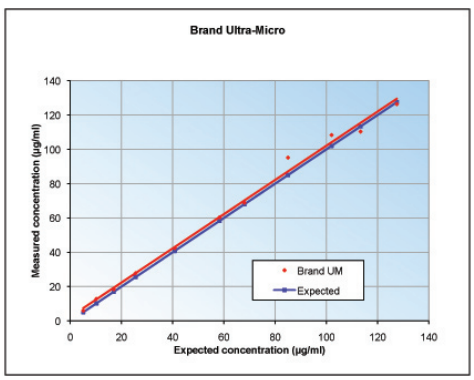

Figure 11. Concentration graph for Brand 759 200. Concentration range 5.1 – 127.5 µg/ml.

The average purity value (Abs_{260}/Abs_{280}) of the different dilutions and cuvette types was ≈ 1.95. This good purity value is as it should be for samples spiked from a pure stock.

To compare quartz and plastic as material, the limit of detection (3SD) was calculated for the quartz Semi-Micro, and the corresponding plastic cuvette (Hellma 104 –QS : 3.8 µg/ml, Brand 759 150: 14.2 µg/ml). As can be seen from the results, there is a visible sensitivity difference between the two materials. However, it is not significant for most quantitation purposes.

The spectral scans were made for several concentrations (dilutions). For the result summary below, one spectrum was chosen for each of the cuvette types. This was made on the basis of the maximum absorbance, which was chosen to be about 1 for each type. All of the DNA spectra were then normalized against this highest absorbance within the spectrum to make them comparable to each other (Figure 12).

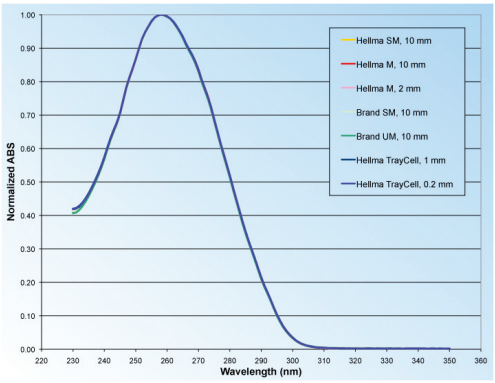

Figure 12. DNA spectra with each cuvette type.

It can be seen from the graph that all of the spectra are almost identical in shape and the spectrum measurement can be made comparably with any of the cuvettes with the Multiskan GO.

Summary

By choosing the right cuvette(s), it is possible to easily cover a wide concentration range in DNA quantitation measurements.

Ready-made DNA calculations and pathlength correction make Multiskan GO a versatile tool for DNA quantitative and qualitative assays. The automatic spectrum is also a valuable tool for fast analysis of the sample quality.

Further information

For further information about the Thermo Scientific Multiskan GO microplate spectrophotometer, please refer to: www.thermoscientific.com/readingroom

For further information about the cuvettes used in this study, please refer to: http://www.hellma-analytics.com or www.brandtech.com.

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

North America:
USA / Canada
+1 800 522 7763

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

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+41 44 454 12 12

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

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