

Photometric DNA quantification using the Multiskan Sky Microplate Spectrophotometer operated with SkanIt Software 5.0

Abstract

This technical note shows how to set up a photometric nucleic acid quantification assay using the Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer operated with Thermo Scientific™ SkanIt™ Software 5.0. Double-stranded DNA (dsDNA) is used as an example to demonstrate the calculation of nucleic acid concentrations.

Background

Photometric quantification by absorbance measurements at 260 nm is extensively used to quickly estimate concentrations of nucleic acids prior to downstream applications. Purified nucleic acids typically produce a Gaussian-shaped absorbance profile, with a maximum peak at 260 nm. The concentration of nucleic acids can be determined from the well-known Beer–Lambert law:

$$c = \frac{A}{\epsilon L} \quad (\text{Equation 1})$$

where:

- A: absorbance measured at 260 nm (with subtraction of background absorbance at 320 nm)
- L: light path or pathlength (cm)
- ϵ : extinction coefficient or intrinsic absorptivity of the nucleic acids, expressed in $(\mu\text{g}/\text{mL})^{-1}(\text{cm})^{-1}$

For easier use of the above equation, it is often written as:

$$c = \frac{A}{L} \times \text{factor} \quad (\text{Equation 2})$$

where:

- factor: corresponds to $1/\epsilon$, expressed in $(\mu\text{g}/\text{mL})(\text{cm})$



For estimation of dsDNA, single-stranded DNA (ssDNA), or RNA concentrations, the factors shown in Table 1 are typically used. These factors are also called “standard coefficients” by other manufacturers. These factors provide reasonable estimates for long sequences of nucleic acids with an even distribution of bases, assuming a pathlength of 1 cm.

Table 1. Factors typically used for photometric calculations of nucleic acids, according to the Beer–Lambert equation.

Nucleic acid	Factor = $1/\epsilon$ (for 1 cm pathlength); $(\mu\text{g}/\text{mL}) \times \text{cm}$
dsDNA	50 (1 absorbance unit = 50 $\mu\text{g}/\text{mL}$)
ssRNA	40 (1 absorbance unit = 40 $\mu\text{g}/\text{mL}$)
ssDNA	33 (1 absorbance unit = 33 $\mu\text{g}/\text{mL}$)

With the Multiskan Sky Microplate Spectrophotometer, nucleic acid concentrations can be measured using several sample platforms, such as the Thermo Scientific™ μ Drop™ Plate, microplates, or cuvettes, covering a wide range of volumes from about 4 μ L up to several milliliters. When changing sample platforms, an important factor to consider is the light pathlength. The pathlength is the distance through which the light has to pass in order to reach the detector. When a sample is measured in a standard spectrophotometer using a cuvette, light passes through the sample horizontally and the light pathlength is fixed, typically at 1 cm. However, when microvolumes are used (either in the μ Drop Plate or in microplates), light passes through the sample vertically and the pathlength is shorter and can, in the case of microplates, vary.

Note: We recommend always performing the calculation of dsDNA concentrations with background subtraction at 320 nm to exclude interference from light scattering caused by particles or precipitates in the samples.

The measured absorbances of samples and blanks in microplates are pathlength-corrected using measurements at 900 and 975 nm. Concentration is calculated using the following equation:

$$c = ((A_{260} - A_{260 \text{ blank}}) - (A_{320} - A_{320 \text{ blank}})) \times 50 \mu\text{g/mL} \quad (\text{Equation 3})$$

where:

A_{260} , $A_{260 \text{ blank}}$: absorbances measured at 260 nm for sample and blanks, respectively (corrected to 1 cm pathlength)

A_{320} , $A_{320 \text{ blank}}$: absorbances measured at 320 nm for sample and blanks, respectively (corrected to 1 cm pathlength)

By following the instructions outlined here, nucleic acid concentrations as well as purities can be easily calculated using SkanIt Software 5.0 with the Multiskan Sky Microplate Spectrophotometer. In addition, simple modifications of this protocol can be introduced to make it applicable for RNA or ssDNA measurements.

Methods

1. dsDNA samples and formats

The samples to be tested with the Multiskan Sky Microplate Spectrophotometer can be measured in microplates, μ Drop Plates, or cuvettes. If desired, you may dilute your samples. For quality control purposes, we recommend using Invitrogen™ UltraPure™ Salmon Sperm DNA (Cat. No. 15632011, 10 mg/mL), with dilutions in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). The general recommendations for various sample formats are listed in Table 2.

Table 2. Sample formats that can be used with the Multiskan Sky spectrophotometer.

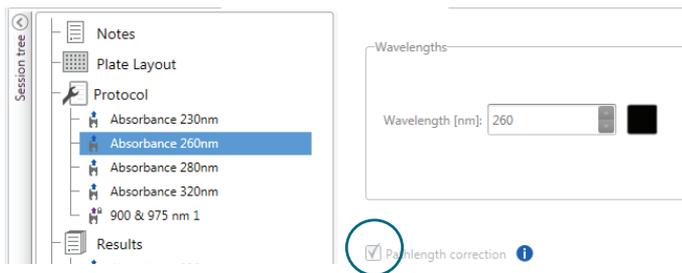
Format	General recommendation
UV-quality 96-well microplates	Use at least 100 μ L per well
UV-quality 96-well half-area microplates	Use at least 40 μ L per well
UV-quality 384-well microplates	Use at least 25 μ L per well
μ Drop Plate	Use at least 4 μ L per well
Cuvettes	Use masked cuvettes, preferably; and Z-height of the cuvette should be 8.5 mm

2. Measurement procedure

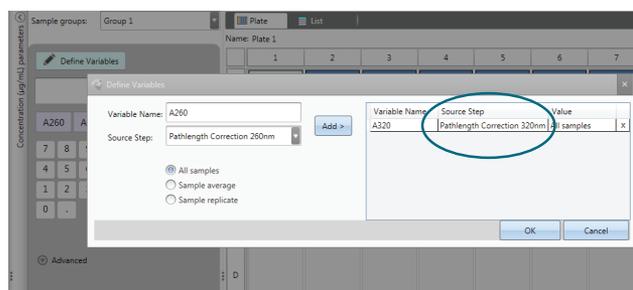
- In this example, 96-well plates (100 μ L sample volume) are used to demonstrate the direct photometric quantification of dsDNA.

2.1. Measurement setup

- Create a new measurement session with SkanIt Software 5.0.
- Select the correct “96 well plate” template in the “Plate Layout” (select “384 well plate”, “ μ Drop Plate”, or “Cuvette”, when appropriate).
- Define the samples in the “Plate Layout”. **Note:** At least one blank well is required for the plastic and buffer background subtraction.
- Add the “Absorbance” measurement steps in the “Protocol” and enter the corresponding measurement wavelengths (230 nm, 260 nm, 280 nm, and 320 nm) (see below). Descriptive naming of the “Protocol” steps (e.g., “Absorbance 260 nm”) facilitates the data analysis. Measurements at 230 nm and 280 nm are used only for the calculations of the purity ratios (A_{260}/A_{230} and A_{260}/A_{280}). **Note:** An alternative to this example protocol step is to add one single “Absorbance” measurement step with “Multiple Wavelengths”.
- Select “Pathlength correction” to activate the automatic pathlength-correction measurements. A step for measurements at 900 and 975 nm will be added automatically to the protocol when it is executed.
- Place the microplate into the reader and select “Start”.



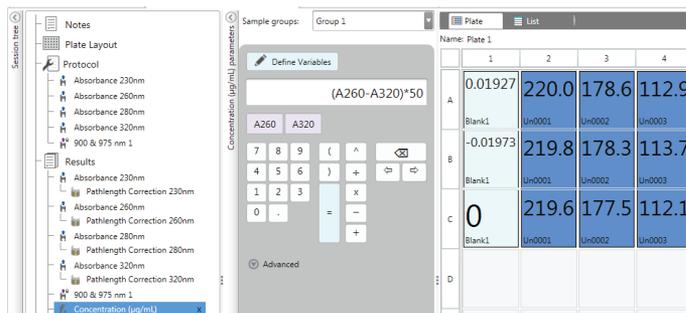
select “Pathlength Correction 320nm” as the “Source Step”. Select “Add” to select the defined variables.



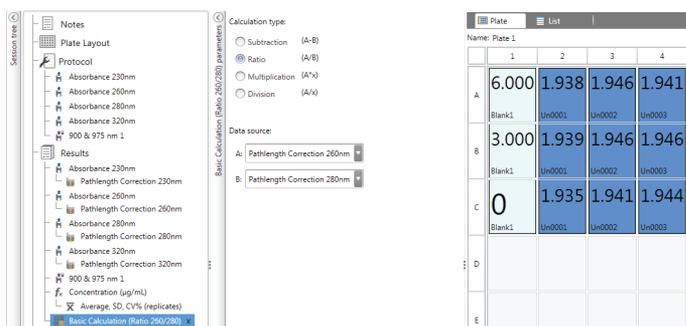
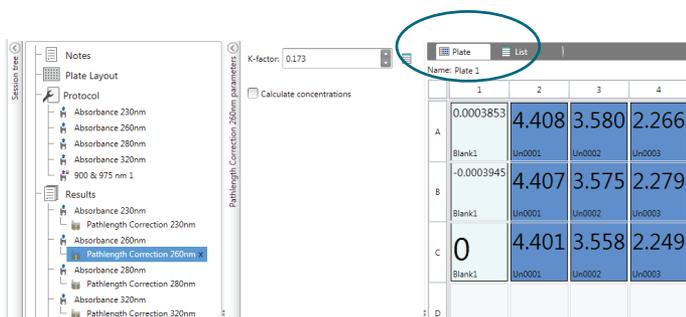
2.2. Results and export

- After the measurement is completed, SkanIt Software 5.0 displays different results in the “Results” view. The “Absorbance” measurement step includes the measurement data of the dsDNA assay wavelengths. The “900 & 975 nm” step includes the data required for “Pathlength correction”.
- The “Results” view will be shown when the measurements are completed.
- Add the “Pathlength Correction” calculation step to each of the “Absorbance” measurement results.
- Select the K-factor to be used for pathlength correction, according to the assay buffer and microplate material. The default value (0.173) is optimal for assays performed in a weak aqueous buffer and plastic plates. (See other considerations related to K-factor in section 3 of this note.)
- The “Pathlength Correction” step calculates the corrected absorbances that correlate to a 1 cm pathlength. **Note:** The “Pathlength Correction” step performs blank subtraction automatically. To see the corrected absorbance values in matrix format, select the “Plate” tab, and to see the data in list format, select the “List” tab.

- Add the equation for calculation of the dsDNA concentrations (equation 3) using the defined variables. The resulting concentration will be expressed in $\mu\text{g/mL}$, the same way it is expressed in the user interface of the Multiskan Sky Microplate Spectrophotometer.



- To calculate “Purity” values, add a “Basic Calculation” step (calculation of “Ratio”). For instance, for A_{260} , select “Pathlength Correction 260nm” as the “Source Step”, and for A_{280} , select “Pathlength Correction 280nm” as the “Source Step”. Select “Add” to choose the defined variables. These purity ratios are similar to the ones reported by the user interface of the Multiskan Sky Microplate Spectrophotometer.



- To calculate the dsDNA concentrations, select “Custom Formula” and define the two variables that are used in equation 3. The variables can be named, for instance, A_{260} and A_{320} . For A_{260} , select “Pathlength Correction 260nm” as the “Source Step”, while for A_{320} ,

Note: If the solution is relatively free of protein, the absorbance at 260 nm can be used as a rough estimation of the dsDNA concentration. (Acceptable A_{260}/A_{280} ratios are between 1.8 and 2.0; see the example above.)

- The measured and calculated data can be easily exported by selecting “Report” in the session tree.
- Define the data items to be included in the results report. You can also select whether to export the data organized in plate format or list format.
- Export the report in the chosen file format (XLS, PDF, TXT, or XML). Alternatively, you can print the report. An automatic export option is given in SkanIt Software 5.0 (see below for an example: “Report” → “Automatic export after execution” → “Save to file”). In this case, we recommend defining the analysis steps before execution.

Automatic export after execution

Print to default printer

Send Excel report via email to:

Save to file: CA

Append

Overwrite

Unique name

{abc}... Browse...

3. Considerations on K-factors

SkanIt Software 5.0 includes predetermined K-factors for commonly used solutions. This makes pathlength correction very simple. If you want to measure the K-factor yourself, add the assay buffer that you are planning to use in your microplate experiments to a cuvette, and measure the absorbance at 975 nm and 900 nm using any photometer capable of measuring at near-IR wavelengths. Make sure the measurement temperature corresponds to the one you will be using in your microplate assay. Then perform a subtraction: $A_{975} - A_{900}$. That value is the K-factor of this assay buffer.

The K-factor should be measured with a polyacrylic plastic UV cuvette with 1 cm optical pathlength. A quartz cuvette should not be used unless the microplate assay is done using a quartz-bottom plate. Quartz and plastic have slightly different behaviors at near-IR wavelengths. **Note:** New K-factors can be added to SkanIt Software 5.0 via “Home” → “Settings” → “K-Factors”.

Conclusions

Using the Multiskan Sky Microplate Spectrophotometer in combination with SkanIt Software 5.0 maximizes versatility in data processing and analysis during photometric quantification of nucleic acids. SkanIt Software 5.0 is optional for use with the instrument models that have the touch-screen interface, and required for operation of the instrument model that lacks the touch-screen interface.

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