Photometric DNA quantification at 260 nm using the Multiskan Sky Microplate Spectrophotometer user interface

Abstract

This technical note shows how to set up a photometric nucleic acid quantification assay using the touch screen user interface of the Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer. 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 an extensively used method 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:

$$c = \frac{A}{\varepsilon L} \qquad \text{(Equation 1)}$$

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 $(\mu g/mL)^{-1}(cm)^{-1}$

The absorption properties of nucleic acids are due to the heterocyclic rings present in all of their five nucleotides and are not associated with their sugar–phosphate backbone. Thus, the absorption of ultraviolet light is a common property of all nucleic acid types (double-stranded and single-stranded DNA as well as RNA). They exhibit varying A_{260}/A_{280} ratios, but in all cases, these values are higher than 1.1. For easier use of the above equation, it is often written as:

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

where:

factor: corresponds to 1/ε, expressed in (µg/mL)(cm)

For estimation of dsDNA, single-stranded DNA (ssDNA), or single-stranded RNA (ssRNA) 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 and 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/ε (for a 1 cm pathlength); (µg/mL)(cm)
dsDNA	50 (1 absorbance unit = 50 µg/mL)
ssRNA	40 (1 absorbance unit = 40 µg/mL)
ssDNA	33 (1 absorbance unit = 33 µg/mL)

With the Multiskan Sky Microplate Spectrophotometer, measurements of nucleic acid concentrations can be made using several sample platforms such as the Thermo Scientific™ µDrop™ Plate, microplates, or cuvettes, covering a wide range of volumes from about 4 µL 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 1 cm. However, when microvolumes are used (either in the µDrop Plate or in microplates), light passes through the sample vertically, and the light pathlength is shorter and, in the case of microplates, variable. In quantifying nucleic acids, the Multiskan Sky Microplate Spectrophotometer automatically corrects for pathlength.

Here we show the steps to set up a measurement protocol for dsDNA through the touch screen user interface of the Multiskan Sky Microplate Spectrophotometer.

Methods

1. dsDNA samples and formats

The samples to be tested with the Multiskan Sky Microplate Spectrophotometer can be measured in microplates, µDrop Plate, 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.

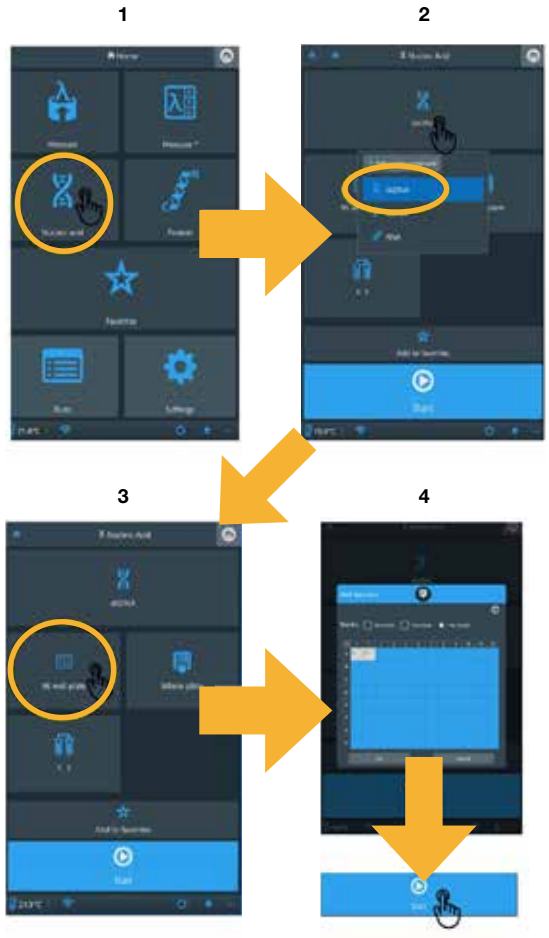
Format	General recommendation
UV-quality 96-well microplates	Use at least 100 µL per well
UV-quality 96-well half-area microplates	Use at least 40 µL per wel
UV-quality 384-well microplates	Use at least 25 µL per well
µDrop Plate	Use at least 4 µL per well
Cuvettes	Use masked cuvettes, preferably; and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

2. Measurement procedure

The user interface of the Multiskan Sky Microplate Spectrophotometer has predefined, built-in protocols that can be used for measurement of dsDNA, ssDNA, RNA, or custom (user-defined) nucleic acids, applicable to all sample formats listed in Table 2. In this example, 96-well plates (100 µL sample volume) are used to demonstrate the direct photometric quantification of dsDNA.

2.1. Measurement setup

- Tap the “Nucleic acid” option on the home screen of the user interface (see panel 1).
- Select dsDNA from the choices (dsDNA, ssDNA, RNA, or custom nucleic acid) in the pop-up menu (see panel 2).
- Select “96 well plate” from the sample format choices (96 well plate, 384 well plate, µDrop Plate, or Cuvette). By tapping the indicated tile (see panel 3), the sample format choices will be similarly displayed in a pop-up window.
- Select the wells to be measured from the plate layout as well as the number of blanks (0, 1, or 2). Please note that the placement of the blanks is fixed according to the selected wells layout (always start from the upper left corner; see panel 4).
- If needed, select the dilution factor of your samples (1:1 is set as default).
- Place the microplate into the Multiskan Sky Microplate Spectrophotometer and tap Start.



2.2. Results calculation and export

- The “Result” view will be automatically shown when the measurements are completed (see panel 5).
- A “Pathlength correction” step will be automatically carried out by the Multiskan Sky spectrophotometer, and the concentrations of nucleic acids will be automatically reported (in µg/mL).
- Purity, expressed as A_{260}/A_{230} and A_{260}/A_{280}, will be automatically calculated and shown on the “Result” screen (see panel 5).
- The spectra (220–350 nm) of each measured sample will be shown in the lowest part of the screen. Results can be viewed as raw absorbance, called “Measurement value” (panel 5), or as “Blank subtracted”, by switching between windows using the small arrowhead to the left of the spectra.
- To export the results, touch the “Export” icon in the upper right corner (see panel 5) and select the format (Microsoft™ Excel™ format, Thermo Scientific™ SkanIt™ software) and the destination location (local network drive, Thermo Fisher Cloud, or USB drive).
- When exporting to the selected location is complete, a green check mark icon will appear for confirmation (see panel 6).

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Note: The calculation of dsDNA concentrations is always performed with a background subtraction at 320 nm. The measured absorbances of samples and blanks (whenever blank samples are included in microplates) will be automatically pathlength-corrected, using measurements at 900 and 975 nm. Concentration is calculated using the following equation:

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

where:
A_{260}, A_{260} blank: absorbances measured at 260 nm for samples and blanks, respectively (corrected to 1 cm pathlength)

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

Conclusions

With the touch screen user interface of the Multiskan Sky Microplate Spectrophotometer, quantification of nucleic acids can be performed with minimal effort and maximum simplicity. The responsive touch screen enables self-explanatory navigation and quick selection of the sample features. Measured absorbances are automatically pathlength-corrected and converted to concentrations for immediate use in downstream applications.

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