

Setting up a Photometric DNA Assay with Thermo Scientific SkanIt Software Using Pathlength Correction

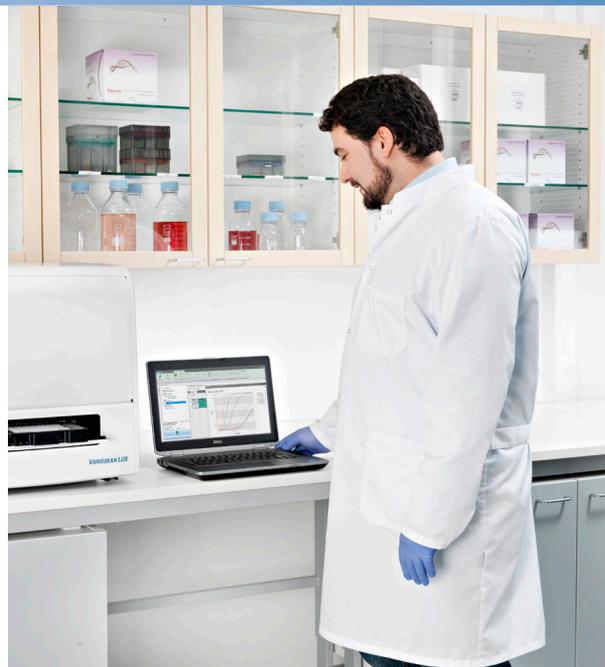
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Abstract

This note shows how to set up a photometric nucleic acid measurement with Thermo Scientific™ Multiskan™ GO microplate spectrophotometer or Thermo Scientific™ Varioskan™ LUX multimode microplate readers using the fourth generation Thermo Scientific™ SkanIt™ software. Double-stranded DNA (dsDNA) is used as an example of calculating nucleic acid concentrations from the sample absorbance measured on a microplate.

As known, $A_{260\text{nm}}$ 1.0 of dsDNA measured at a 1 cm pathlength corresponds to a dsDNA concentration of 50 µg/ml. However, on a microplate the pathlength is never exactly 1 cm, and therefore a pathlength correction method is needed to be able to use this multiplication factor.

SkanIt software has automatic pathlength correction functionality, which normalizes absorbance values measured on a microplate to correspond with absorbance values measured at a 1 cm pathlength (standard cuvette). Pathlength correction enables DNA/RNA concentration calculation directly from absorbance values.



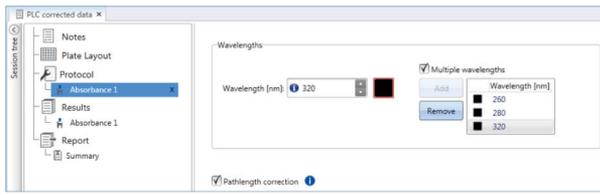
I) DNA assay plate

Add DNA samples and blanks to a UV-quality microplate. You may dilute your samples to conserve them.

- 96-well plates at least 100 µl per well
- 96-well half area plates 40 µl per well
- 384-well plates at least 25 µl per well

II) Measurement setup

1. Create a new measurement session with SkanIt software 4.x.
2. Select the correct 96- or 384-well plate template in the *Plate Layout*.
3. Define the samples in the *Plate Layout*. Note: At least one blank well is required for the plastic and buffer background subtraction.
4. Add the *Absorbance Measurement* step in the *Protocol* and enter the measurement wavelengths (for example 260 nm, 280 nm and 320 nm).
5. Select *Pathlength Correction* to activate the automatic pathlength correction measurements.

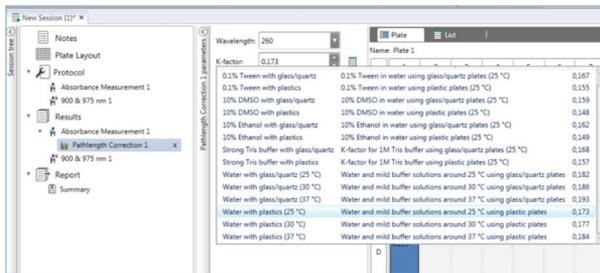


6. Place the microplate into the reader and click *Start*.

III) Performing pathlength correction

After the measurement the SkanIt software displays two result steps in the *Results* view. The Absorbance step includes the measurement data of the DNA assay wavelengths. The 900 & 975 nm step includes the data required for the Pathlength Correction.

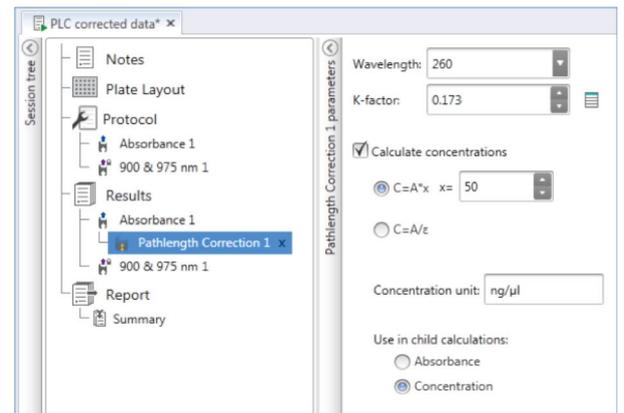
1. Add the *Pathlength Correction* calculation step to the Absorbance measurement results and select 260 nm as the data source.
2. Select the K-Factor to be used for pathlength correction according to the assay buffer and microplate material. The default value, 0.173, is optimal for assays performed in a weak aqueous buffer and plastic plate.



The *Pathlength Correction* step now calculates the corrected absorbance (260 nm) values that correlate to 1 cm pathlength. Note that the Pathlength Correction step performs blank subtraction automatically. To see the corrected absorbance values in matrix format click the *Plate* tab; to see the data in list format click the *List* tab.

IV) Calculating DNA concentrations

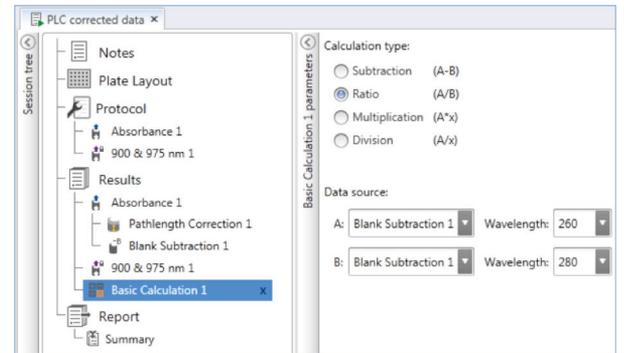
1. In the *Pathlength Correction* step select *Calculate Concentrations* and enter the concentration factor (x), which the corrected absorbance values should be multiplied by (e.g., x = 50 for dsDNA).



The plate view of the results shows the calculated concentrations. The list view shows both the calculated concentrations and the corrected absorbance values.

V) Calculating the 260 nm/280 nm ratio for purity analysis

1. Add the *Blank Subtraction* step to the *Absorbance Measurement* step.
2. Add the *Basic Calculation* step and select *Ratio (A/B)* as the calculation type, blank subtraction as the data source, and the wavelengths A = 260, B = 280.



The results of the Abs_{260}/Abs_{280} ratio calculation are shown both in the *Plate* and *List* view.

VI) Exporting results

Measurement and calculated data can be easily exported in the *Report* view.

1. Click on the *Report* in the session tree.
2. Define the data items to be included in the result report. You can also select whether to export the data organized in plate or list format.
3. Export the report in the chosen file format (Excel, PDF, text or xml). Alternatively you can print the report.

K-Factor

SkanIt software includes pre-determined K-Factors for commonly used solutions. This makes pathlength correction very simple.

If you want to measure the K-Factor yourself, add assay buffer to a cuvette and measure the absorbance at 975 nm and 900 nm using any photometer capable of measuring at near-IR area. Then perform a subtraction

$A_{975\text{ nm}} - A_{900\text{ nm}}$. That value is the K-Factor of the buffer.

The K-Factor should be measured with a polyacrylic plastic UV-cuvette with 10 mm optical pathlength. Quartz cuvette should not be used unless the microplate assay is also done using a quartz bottom plate. Quartz and plastic have slightly different behavior at near-IR area. New K-Factors can be added to SkanIt software via Home -> Settings -> K-Factors.

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