Thermo Labsystems

Multiskan® Spectrum Microplate Spectrophotometer

TECHNICAL NOTE

NUCLEIC ACID QUANTITATION

INTRODUCTION

Nucleic Acid quantitation is one of the most commonly performed applications of UV spectrometry. The Thermo Labsystems Multiskan® Spectrum Microplate Spectrophotometer is a contemporary system that can rapidly acquire, process and report data with a high degree of precision and a minimum of user interaction.

Nucleic Acid in biological samples can be measured at 260 nm. The absorbance can be readily related to the concentration and the calculation can be automated to report the actual nucleic acid concentration.

Each common form of nucleic acid (dsDNA, ssDNA, and RNA) has a commonly used factor by which absorbance at 260 nm can be multiplied to estimate the concentration of that nucleic acid. A factor of 50 is commonly used for dsDNA, 33 for ssDNA, and 40 for RNA. For example, for pure dsDNA in solution, multiplying the 260 nm absorbance by 50 yields an estimate of dsDNA concentration in µg/mL.

MATERIALS AND METHODS

Double stranded DNA from calf thymus, Sigma D-3664, was diluted to contain 1 mg/mL DNA in Tris-EDTA buffer, pH 7.5 (TE buffer). Dilutions to prepare solutions between 0.2 and 200 µg DNA per mL were performed.

200 µl of each dilution in eight replicates was added to the wells of a UV transparent 96-well microplate (Greiner UVStar® 96) while 60 µl was added to a UV transparent 384-well microplate (Greiner UVStar 384).

Absorbance was measured via a Thermo Labsystems Multiskan Spectrum Microplate Spectrophotometer at λ = 260 nm with pathlength correction using microplates and microcuvettes. Standard cuvette measurements were made with a standard spectrophotometer.

DNA QUANTITATION

The absorbance of various DNA samples was measured via a standard spectrophotometer using a 1 cm^2 cuvette and with the Multiskan Spectrum using a microcuvette as well as 96 and 384 well microplates at 260 nm. Eight replicates of each sample were measured in UV transparent microplates. The *Pathlength Correction* was utilized and the DNA concentration calculated using Equation 1. The results are presented in Table 1.

$$\text{DNA concentration } (\mu g/ml) = 50 * A_{260nm} \qquad 1$$

Table1. DNA quantitation using the Multiskan Spectrum Microplate Spectrophotometer

Standard Spectro Cuvette	Multiskan Spectrum Microcuvette	Multiskan Spectrum 96-well plate		Multiskan Spectrum 384-well plate	
µg/mL	µg/mL	µg/mL	CV% (n=8)	µg/mL	CV% (n=8)
11.55	11.85	11.71	1.78	12.37	5.7
23.20	23.65	23.45	0.83	24.33	2.14
46.00	46.40	46.33	0.79	47.39	1.17
90.80	86.40	91.09	0.69	92.67	0.85

We note very good correlation between pathlength corrected microplate data and data from cuvettes. In addition, we can measure more concentrated nucleic acid solutions in the microplate without dilution (due to shorter pathlength) but still use the "standard" factor used with 1 cm cuvettes.

Data Processing

Two very important Data Processing routines are Pathlength Correction and the automated calculation of the concentration. Dialog boxes are provided to lead the operator through the procedure to set up these features.

Pathlength Correction

When a microplate is used for quantitative analysis, there may be small differences in the pathlength because of differences in pipetting of the sample and reagents. These changes will create errors in the measured absorbance of the samples (since a microplate involves a "straight through the well measurement"). Pathlength correction involves measuring the absorbance of the wells at a wavelength that is not of analytical interest to determine the precise pathlength, then using the pathlength at the analytical wavelength to correct for small experimental errors.

Generating pathlength correction is performed by simply following a series of steps presented by the instrument. Once the correction factor is generated, it is stored in memory and can be recalled as desired via the Reader Protocol dialog box (Figure 2) by enabling Pathlength Correction and selecting the appropriate solvent.

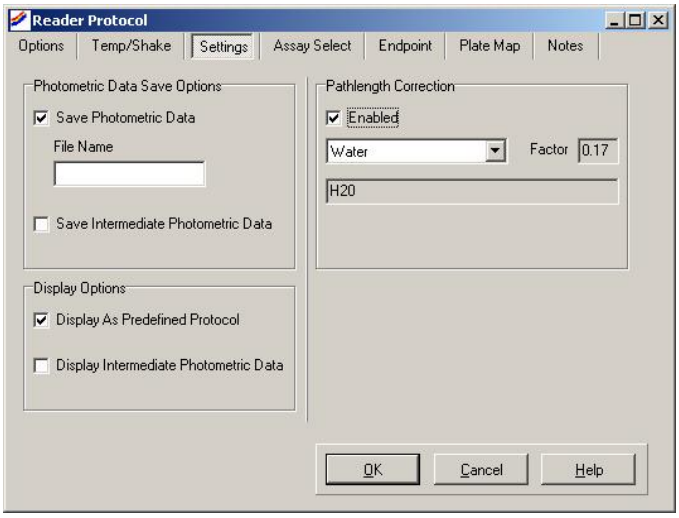

Figure 2. Enabling Pathlength Correction

Automated Calculation of the Concentration

The Multiskan Spectrum software includes the ability for the user to generate the result on an automated basis using the *Create Universal Equation* dialog box (Figure 3).

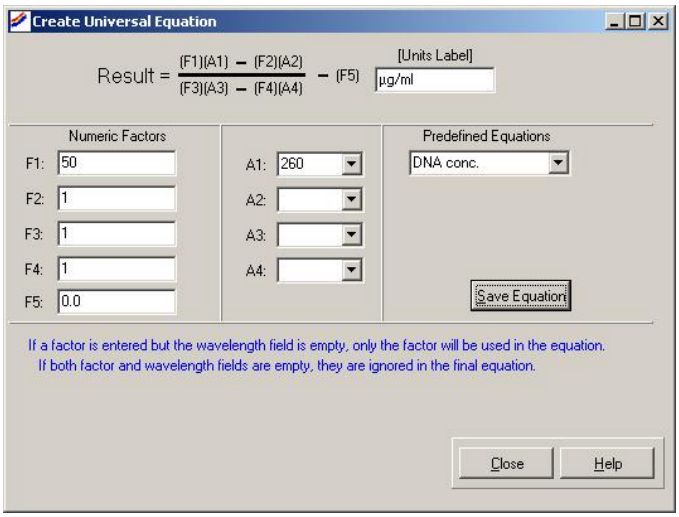

Figure 3. The Create Universal Equation Dialog Box

This dialog box lets the user indicate the equation to be used to calculate the concentration of the analyte. Four analytical wavelengths, four multiplication (division) factors and the addition (subtraction) of a constant can be used. The calculation is automatically performed and reported.

Since the "standard" factors typically used for nucleic acid quantitation (50, 33, and 40) are based on molar absorbtivities of "standard" nucleic acids (typical base composition %) the factors are really "one size fits all" approximations. If the researcher knows enough about the nucleic acids they are working with, they can use this universal equation editor to define a customized factor based on a better knowledge of the typical molar absorbtivity of these particular nucleic acid samples.

Conclusions

This technical note clearly indicates that the Multiskan Spectrum Microplate Spectrophotometer is capable of providing quantitative analysis of DNA using either cuvettes or microplates. The system includes a number of advanced features to ensure a high degree of analytical precision to simplify data reporting and processing.

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