



Low Volume Nucleic Acid Quantification using a Multi-Volume Spectrophotometer System

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Micro-volume quantification allows for rapid assessment of concentrations of nucleic acid. The Epoch™ Multi-Volume Spectrophotometer System provides the ability to measure a wide range of samples from 2 μ L to mL volumes, using the Take3™ Multi-Volume Plate. Here we demonstrate the utility of Epoch and Take3 for dsDNA quantification and compare accuracy to gold standard techniques.

Introduction

The quantification of nucleic acids is a necessary procedure after isolation from samples such as tissues, cells or body fluids. Downstream applications include PCR, RT-PCR, sequencing, restriction digestions and ligations. All these applications involve enzymatic reactions where efficiency is dependent on the relative concentrations of nucleic acid, enzyme and other reactants, hence the need for quantification. Amounts of nucleic acid isolated from most kits can range anywhere from ng to μ g and are typically eluted in 10 to 100 μ L volumes. Nucleic acid concentrations can range from sub ng/ μ L to thousands of ng/ μ L. Spectrophotometry is a very popular method for nucleic acid quantification as it is a simple, accurate and non-destructive method for the measurement of nucleic acid over much of the range of concentrations described above. For standard 1 cm pathlength cuvettes, dilution of the sample is typically required for nucleic acid concentrations above about 100 – 200 ng/ μ L to avoid Beer's Law non-linearity issues at high optical density. Here we describe the Epoch™ Multi-Volume Spectrophotometer System that allows the user to rapidly measure from 1 -16 samples in 2 μ L volumes without any need for dilution. The unique Take3™ plate included in the System also provides the capability to read BioTek's BioCell™ or any standard 1 cm pathlength cuvette. Finally, the Epoch can be used as a standard monochromator-based microplate spectrophotometer by replacement of the Take3 plate with any standard 6- to 384-well density microplate for many other applications.

Materials and Methods

The Epoch™ Multi-Volume Spectrophotometer System (Figure 1) is a flexible spectrophotometric instrument for reading 6- to 384-well microplates, micro-volume sample quantification (2 μ L), 1 cm pathlength direct measurements using BioTek's BioCell or any standard spectrophotometric cuvette using the Take3 plate (Figure 2).



Figure 1. The Epoch™ Multi-Volume Spectrophotometer System incorporating the Epoch™ Spectrophotometer and, Take3™ Multi-Volume Plate.



Figure 2. Take3™ plate with 16 microspots (2 μ L) for micro-volume measurements. The plate can also accommodate 2 BioCells, and a standard 1 cm pathlength cuvette.

The Take3™ plate has a standard SBS footprint and has 16 microspots arranged as columns akin to columns 2 and 3 of a 96-well microplate. 2 μ L volumes of a sample can be pipetted into individual microspots or a multichannel pipette may be used to load eight samples simultaneously.

Methods

All double-stranded DNA (dsDNA) standards were created by serial dilution of a concentrated stock of herring sperm dsDNA in TE buffer (10 mM TRIS, 1 mM EDTA, pH=7.0). Take3 micro-volume data was obtained with undiluted standard samples. Each standard concentration was loaded 5-times at each microspot location on the Take3 plate using an 8-channel manual pipettor and absorbances read at 260, 280 and 320 nm. BioCell data was acquired using either undiluted or, for higher concentration samples, a 20-fold dilution of standard in TE. All sample measurements were background corrected using a TE buffer blank at 260 nm. All concentrations depicted are based on a 1 cm pathlength and 50 ng/ μ L/OD.

Results and Discussion

Reproducibility across 16 Microspots in the Take3 Plate

Figure 3 demonstrates the DNA concentrations obtained using the micro-volume feature of the Take3 Multi-Volume Plate to measure a 2050 ng/ μ L dsDNA standard. This represents the typical upper end of the DNA concentration range obtained from DNA plasmid isolation kits such as Invitrogen's PureLike HiPure maxi kit. The optical density obtained for this undiluted dsDNA sample averaged 1.8 ODs for each of the micro-volume microspots. With a 1 cm pathlength this undiluted sample would produce a theoretical OD of approximately 36, assuming the Beer's law was still obeyed. The %CV across the 16 microspots was 1.8%. The % relative standard deviation across the 16 microspots is 1.8%.

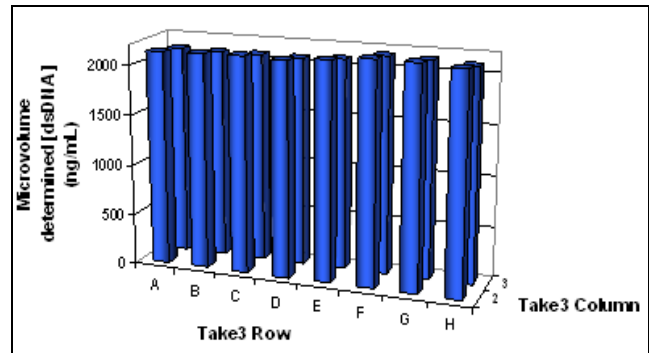


Figure 3. dsDNA concentration determinations of a 2050 ng/ μ L herring sperm dsDNA sample using all 16 microspots of a Take3 plate.

Accuracy using 16 Microspots of a Take3 Plate

The accuracy of micro-volume quantification was determined across a broad range of herring sperm dsDNA concentrations. The replicate measurements from the 16 microspots were used to determine average concentrations and the standard deviation was used as vertical error bars in Figure 4, below.

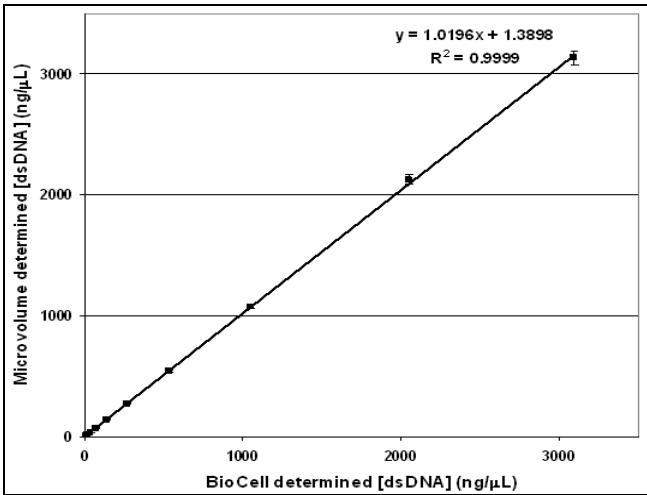


Figure 4. Accuracy of micro-volume quantification of dsDNA relative to measurements made at 1 cm path length with BioCell™ accessory. Both micro-volume and BioCell measurements are made with the Take3™ Multi-Volume Plate.

It is apparent from the slope of the graph in Figure 4 that there is a 2% difference in accuracy across the broad range of dsDNA concentrations between undiluted micro-volume determinations and 1 cm pathlength determinations made with the BioCell.

Conclusions

The Epoch Multi-Volume Spectrophotometer System is a unique laboratory instrument that serves as a micro-volume quantitative spectrophotometer; a cuvette-based spectrophotometer for the highest accuracy absorbance measurements; and finally as a monochromator-based scanning microplate absorbance reader for performing ELISAs and other colorimetric assays.

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