

Low Volume Nucleic Acid Quantification using A Multi-Volume Microplate Spectrophotometer System



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Overview

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 µg and are typically eluted in 10 to 100 µL volumes. Nucleic acid concentrations can range from sub ng/µL to thousands of ng/µL. 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/µL 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 µL 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 & Methods

dsDNA Standards

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/µL/OD.

dsDNA from Mesothelioma Cells

Genomic DNA was prepared from three individual samples of ~1 million mesothelioma cells using an AllPrep DNA/RNA/Protein Mini Kit (Qiagen). Briefly, cells were collected by centrifugation, washed and stored at -80°C until needed. The cells were thawed at room temperature, washed one additional time to remove any residual media and resuspended in 600 µL RTL lysis buffer (supplied with kit). Cells were then homogenized via passage through a syringe fitted with a 20 gauge needle 5-10 times and subject to purification per the AllPrep protocol in the handbook. DNA was eluted from the DNA binding column in 100 µL of elution buffer (supplied with kit) preheated to 70°C. The DNA was then quantified by absorbance spectroscopy at 260 nm utilizing the Epoch™ /Take3™ Multi-Volume Spectrophotometer System (BioTek Instruments, Inc.) and the NanoDrop 2000c (Thermo Scientific).

All measurements were accomplished using 2 µL sample volumes. Briefly, the three unknown samples were loaded in triplicate on the Take3™ plate in well locations A2-F3 and read simultaneously using Gen5™ software and the Epoch™ reader. The plate was loaded 3 times resulting in 9 replicate measurements of each sample. Samples were loaded and read individually in triplicate on the NanoDrop 2000c.

BioTek Instrumentation

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 µL), 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 µL) 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 µL volumes of a sample can be pipetted into individual microspots or a multichannel pipette may be used to load eight samples simultaneously.

Reproducibility

Figure 3 demonstrates the DNA concentrations obtained using the micro-volume feature of the Take3™ Multi-Volume Plate to measure both a 36 ng/µL and a 2050 ng/µL dsDNA standard. The former concentration represents the typical lower end of genomic DNA isolation kits and the latter concentration is representative of the upper end of the DNA concentration range obtained from DNA plasmid isolation kits. The optical density obtained for these undiluted dsDNA samples averaged 0.075 and 1.8 ODs, respectively for each of the micro-volume microspots. With a 1 cm pathlength the latter 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 2.3 and 1.8%, respectively.

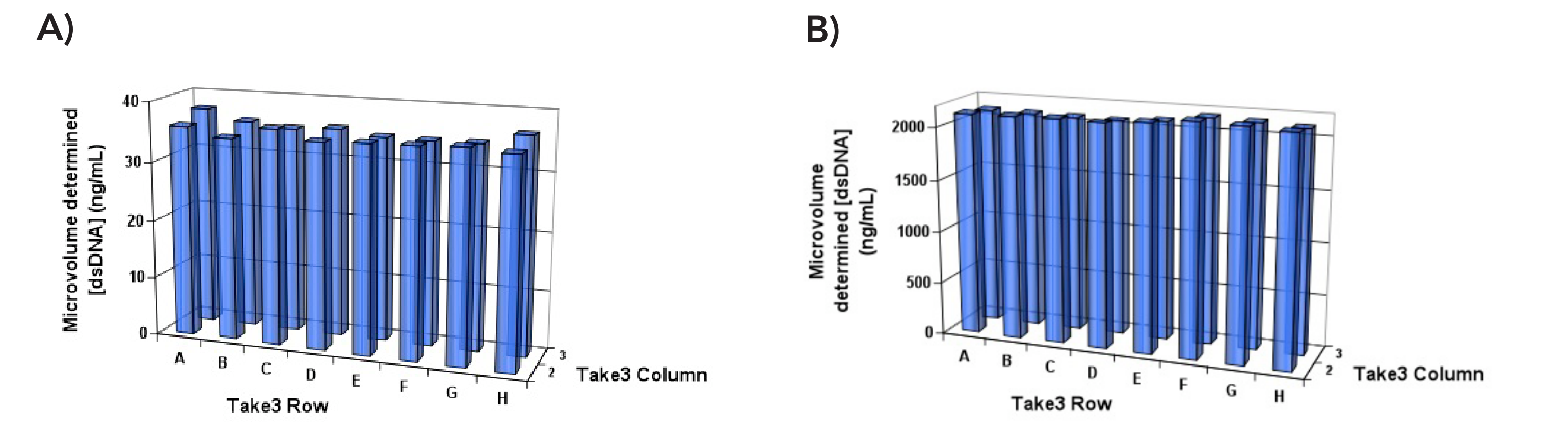


Figure 3 – dsDNA concentration determinations of a A) 36 ng/mL and B) 2050 ng/µL herring sperm dsDNA sample using all 16 microspots of a Take3™ plate.

Accuracy vs 1 cm Path Length

The accuracy of micro-volume quantification relative to 1 cm path length quantification in BioTek's BioCell™ 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.

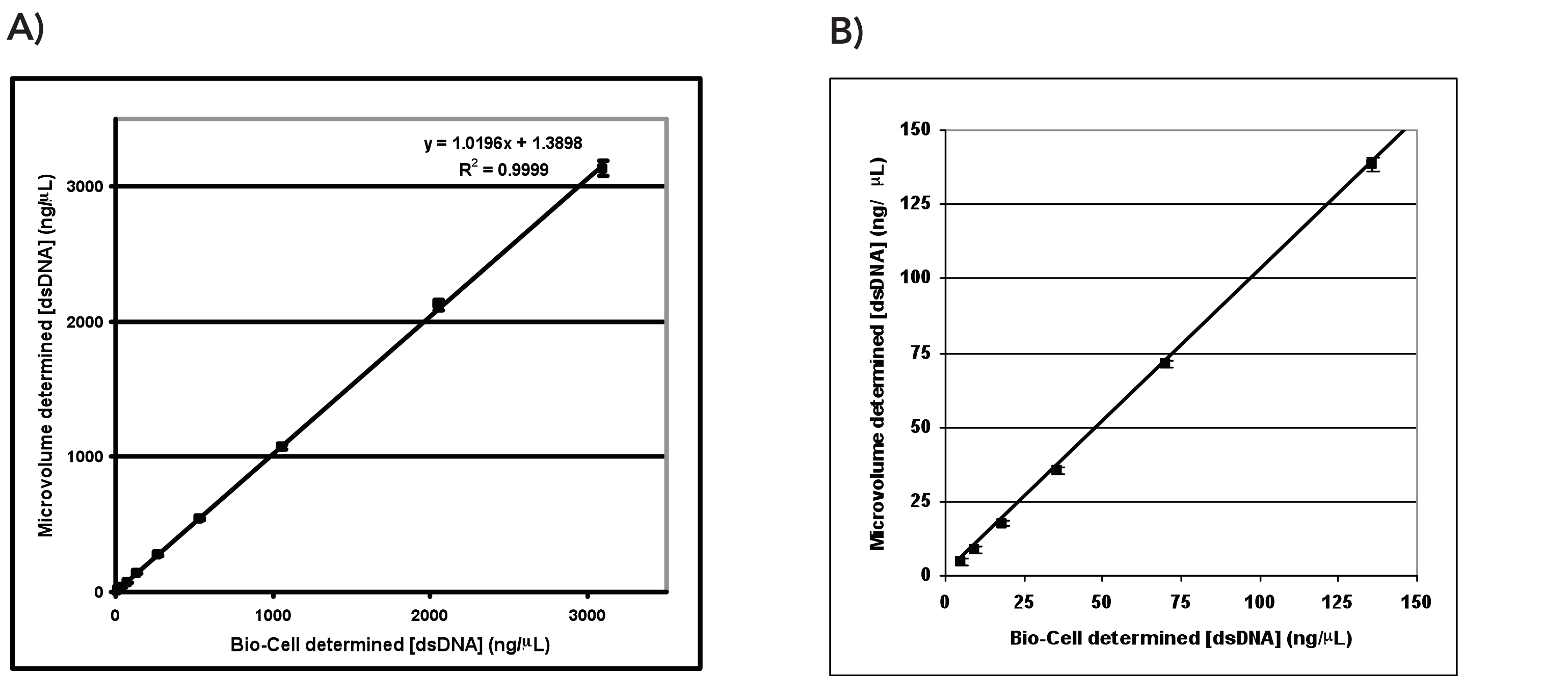


Figure 4 – Accuracy of micro-volume quantification of dsDNA relative to measurements made at 1 cm path length with BioCell™ accessory. Both micro-volume and BioCell™ measurements are made with the Take3™ Multi-Volume Plate. A) Full [dsDNA] range from 4 - 3100 ng/µL; B) Reduced view of same data to [dsDNA] range from 4 - 130 ng/µL

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™.

Accuracy vs Nanodrop, Mesothelioma Cells

Table 1 compares the quantification of approximately 10⁶ mesothelioma cells using both Epoch™ / Take3™ and Nanodrop 2000c.

Trial #	[dsDNA] (ng/µL)	
	Epoch / Take3	Nanodrop 2000c
1	154 ± 2	151 ± 4
2	246 ± 2	245 ± 3
3	105 ± 1	105 ± 2

Table 1 – dsDNA concentration determinations from ~10⁶ mesothelioma cells. The quantification was performed on three separate cell pellets of differing cell number/pellet. Error provided is at a 95% confidence level from replicate measurements.

It is apparent that there is no statistical difference between the readings from Epoch™ / Take3™ and Nanodrop 2000c. The slightly lower error apparent in the Epoch™ / Take3™ data is due to the relative ease in performing large numbers of replicates using the 16 available micro-wells.

Conclusions

1. Take3™ allows microvolume nucleic acid quantification to 2 µL volumes.
2. Nominal path length of 0.5 mm obviates the need for sample dilution.
3. The provision of 16 micro-wells allows for highly accurate determinations with low error.
4. Accuracy is comparable to gold standard techniques based on a 1 cm path length.
5. There is no statistical difference between quantification by Epoch™ / Take3™ and Nanodrop 2000c.
6. Epoch™ can be used with standard microplates (6- to 384-well densities) for a myriad of other applications.