Low-Volume Nucleic Acid Quantification Using a Multivolume Microplate Spectrophotometer System

Peter Brescia, Lenore Buehrer, and Gary Barush
Agilent Technologies, Inc.

Agilent
Trusted Answers

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 depends 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 nondestructive method for the measurement of nucleic acid at the concentrations previously described. For standard 1-cm pathlength cuvettes, dilution of the sample is typically required for nucleic acid concentrations above 100 to 200 ng/µL to avoid Beer's law nonlinearity issues at high optical density. Described here is the Agilent BioTek Epoch microplate spectrophotometer system that allows the user to rapidly measure from 1 to 16 samples in 2 µL volumes without any need for dilution. The unique Agilent BioTek Take3 microvolume plate included in the system also provides the capability to read the Agilent BioTek BioCell or any standard 1-cm pathlength cuvette. Finally, the Epoch microplate spectrophotometer can be used as a standard monochromator-based microplate spectrophotometer by replacement of the Take3 microvolume plate with any standard 6- to 384-well density microplate for many other applications.

Materials and 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 microvolume plate data was obtained with undiluted standard samples. Each standard concentration was loaded five 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 a Qiagen AllPrep DNA/RNA/Protein Mini Kit. Briefly, cells were collected by centrifugation, washed and stored at –80 °C until needed. The cells were thawed at room temperature, washed one more 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 to 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 using the Agilent BioTek Epoch microplate spectrophotometer with the Agilent BioTek Take3 microvolume plate and the Thermo Scientific NanoDrop 2000c spectrophotometer.

All measurements were accomplished using 2 µL sample volumes. Briefly, the three unknown samples were loaded in triplicate on the Take3 microvolume plate in well locations A2 to F3 and read simultaneously using Agilent BioTek Gen5 microplate reader and imager software and the Epoch microplate spectrophotometer. The plate was loaded three times resulting in nine replicate measurements of each sample. Samples were loaded and read individually in triplicate on the Thermo Scientific NanoDrop 2000c spectrophotometer.

Instrumentation

The Agilent BioTek Epoch microplate spectrophotometer (Figure 1) is a flexible spectrophotometric instrument for reading 6- to 384-well microplates, microvolume sample quantification (2 µL), 1-cm pathlength direct measurements using the Agilent BioTek BioCell or any standard spectrophotometric cuvette using the Agilent BioTek Take3 microvolume plate (Figure 2).

Figure 1. The Agilent BioTek Epoch microplate spectrophotometer system incorporating the Epoch microplate spectrophotometer and Take3 microvolume plate.

Figure 2. The Agilent BioTek Take3 microvolume plate with 16 microspots (2 µL) for microvolume measurements. The plate can also accommodate two BioCells, and a standard 1-cm pathlength cuvette.

The Take3 microvolume plate has a standard SBS footprint and has 16 microspots arranged as columns akin to columns two and three 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 microvolume feature of the Agilent BioTek Take3 microvolume plate to measure both a 36 ng/µL and a 2,050 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 (OD) for these undiluted dsDNA samples averaged 0.075 and 1.8 ODs, respectively for each of the microvolume microspots. With a 1-cm pathlength, the latter undiluted sample would produce a theoretical OD of approximately 36, assuming Beer's law was still obeyed. The %CV across the 16 microspots was 2.3 and 1.8%, respectively.

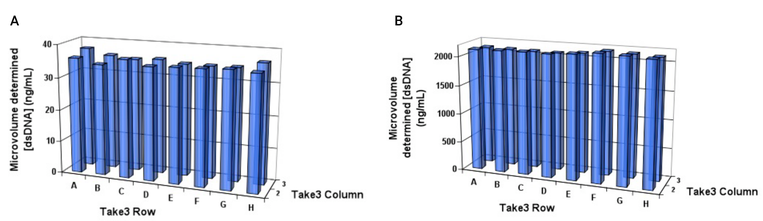

Figure 3. dsDNA concentration determinations of A) 36 ng/mL and B) 2,050 ng/µL herring sperm dsDNA sample using all 16 microspots of an Agilent BioTek Take3 microvolume plate.

Accuracy versus 1-cm pathlength

The accuracy of microvolume quantification relative to 1-cm pathlength quantification in the Agilent BioTek 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.

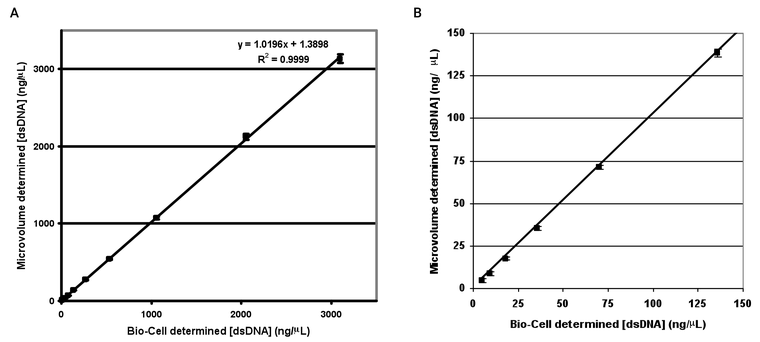

Figure 4. Accuracy of microvolume quantification of dsDNA relative to measurements made at 1-cm pathlength with Agilent BioTek BioCell adapter. Both microvolume and BioCell measurements are made with the Agilent BioTek Take3 microvolume plate. (A) Full [dsDNA] range from 4 to 3,100 ng/µL; (B) Reduced view of same data to [dsDNA] range from 4 to 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 microvolume determinations and 1-cm pathlength determinations made with the Agilent BioTek BioCell.

Accuracy versus competitive spectrophotometer

Table 1 compares the quantification of approximately 10^6 mesothelioma cells using both Agilent BioTek Epoch/Agilent BioTek Take3 and Thermo Scientific NanoDrop 2000c spectrophotometer.

	[dsDNA] (ng/µL)	
Trial Number	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 ~10^6 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 the Agilent BioTek Epoch microplate spectrophotometer with Agilent BioTek Take3 microvolume plate and the Thermo Scientific NanoDrop 2000c spectrophotometer. 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 microwells.

Conclusion

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

www.agilent.com/lifesciences/biotek

DE44365.3065393518

Printed in the USA, July 13, 2021
5994-3253EN