Application Note

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Analytical Performance of Nucleic Acid Microvolume Quantification Using the Epoch Spectrophotometer System

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Abstract

Nucleic acids such as DNA and RNA are derived from a variety of biological sources for potential use in downstream applications such as sequencing and gene expression analysis. This application note demonstrates the analytical performance of the Agilent BioTek Epoch spectrophotometer system and especially the Agilent BioTek Take3 multivolume plate with regard to linear dynamic range, limit of detection, precision, and accuracy using microvolume samples to verify its broad applicability to nucleic acid quantification. Comparative data were derived on an Epoch spectrophotometer system and a NanoDrop; performance was shown to be equivalent.

Introduction

Accurate determination of molecular concentrations is prerequisite to the use of purified nucleic acids for a multitude of downstream applications. Quantification is routinely accomplished by spectrophotometric analysis at 260 nm for nucleic acids in a UV transparent vessel. Measurements were historically made with quartz cuvettes that have a fixed pathlength of 1 cm and are typically associated with high precision and accurate measurements. The Epoch spectrophotometer system is capable of accurately measuring up to 16 samples with volumes as low as 2 µL (Figure 1) using the Take3 multivolume plate. The Take3 plate provides a nominal 0.5 mm pathlength allowing measurement over a broad range of concentrations. Concentrations can be measured from dilute, low ng/µL samples as well as samples in the 1000's of ng/µL range. This wide concentration range is typical of the yields from current nucleic isolation methods – yield is dependent on sample type, size, and what sort of nucleic acid is being isolated. The Take3 plate is also capable of accommodating two Agilent BioTek BioCells and a standard cuvette extending the range of quantifiable sample concentrations to sub ng/µL by the extension of pathlength to 1 cm.

This application note shows the analytical performance of the Epoch spectrophotometer system for microvolume analysis in terms of detection limit, linear dynamic range, precision, and accuracy for both dsDNA and RNA.

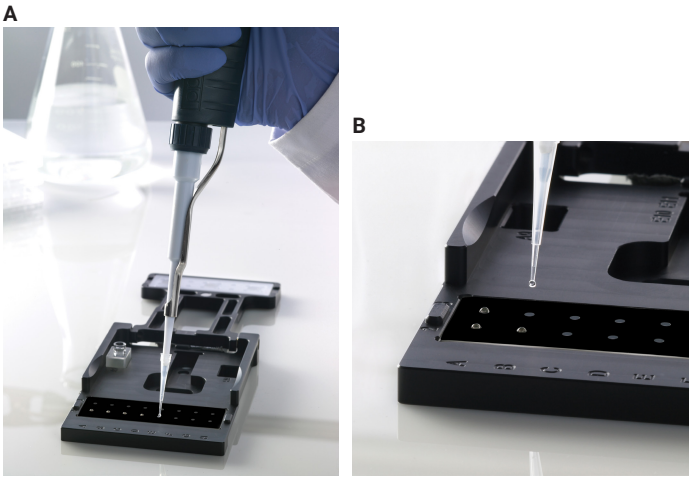

Figure 1. (A) Agilent BioTek Take3 multivolume plate shown with BioCell in place. Two additional locations are available for a second BioCell and a standard cuvette. Sample volumes as low at 2 µL can be loaded on 16 microspot locations using either a single or multichannel pipettor.

Material and methods

Linear dynamic range

All double-stranded DNA (dsDNA) and RNA standards were created by preparing a 1:2 serial dilution series of a concentrated stock of herring sperm dsDNA or yeast (*Saccharomyces cerevisiae*) RNA, respectively, in TE buffer (*tris*-EDTA, pH = 7.0). Epoch spectrophotometer system microvolume data were obtained with undiluted standard samples using the Take3 plate. The Take3 plate was calibrated prior to use to determine pathlength correction values for each microspot. Each standard concentration was loaded 5 times at each microspot location on the Take3 plate using an 8-channel manual pipettor. Optical densities were read at 260, 280, and 320 nm resulting in 80 replicate measurements on each instrument. BioCell data were acquired using either undiluted or, for higher concentration samples, a 20-fold dilution of standard in TE or MilliQ water. NanoDrop microvolume data were determined from replicate measurements of the same nucleic acid standards. All sample measurements were background corrected using a TE buffer blank or MilliQ water, where appropriate. All concentrations depicted are based on a 1 cm pathlength and 50 ng/µL/OD for DNA; 40 ng/µL/OD for RNA.

Detection limit

Limit of detection is typically defined as the analyte concentration that can provide a signal that is three-fold higher than the noise (standard deviation) of the background signal. The standard deviation in the blank signal for each microspot of the Take3 plate was determined from 10 measurements of reloaded blank solution. As with both DNA and RNA measurements made in the Linear Dynamic Range determinations outlined above, 260 nm signals were corrected bichromatically at 320 nm.

Results and discussion

Linear dynamic range and accuracy

dsDNA

Herring sperm dsDNA standards were prepared as a 12-point 1:2 serial dilution series resulting in concentrations ranging from ~4 to 3,000 ng/µL. Microvolume measurements using both Take3 and NanoDrop were compared to those taken using the BioCell placed in the Take3 plate and read on the Epoch microplate reader and appear in Figure 2. It is apparent that the linear dynamic range of the Epoch spectrophotometer system for microvolume analysis is three orders of magnitude, which adequately covers the yield and concentration ranges of most DNA isolation kits (Table 1).

Linear regression analysis was also performed on the data presented in Figure 2. Both microvolume determinations demonstrated near perfect straight line correlations (R^2 ≥0.9998) and slopes of 1.020 and 0.967 for Epoch spectrophotometer system and NanoDrop, respectively. The slope can be used as a measure of accuracy relative to 1 cm pathlength measurements across the [DNA] range presented in Figure 2. Thus Epoch spectrophotometer system shows an average percent difference of 2.0% relative to 1 cm pathlength determinations across three orders of magnitude [DNA]; NanoDrop, 3.3%.

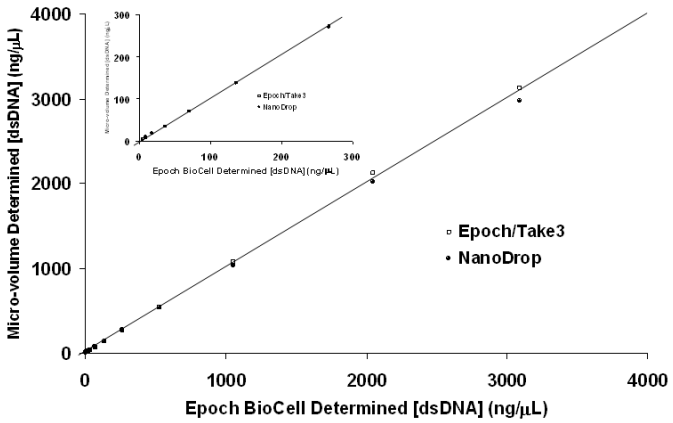

Figure 2. dsDNA standard curve using dilutions of a purified herring sperm sample. Abscissa data is considered actual [dsDNA] as measurements were conducted with 1 cm pathlength using BioCell. Ordinate data are microvolume determinations using an Agilent BioTek Epoch spectrophotometer system and NanoDrop. The straight line is through the origin and has a slope of 1.000, which is considered a perfect equivalence between microvolume and BioCell data. Inset is an exploded view of low dsDNA concentrations.

Table 1. Expected [DNA] in isolate from some commonly used commercially available DNA isolation kits. Yields are provided as expected results from supplier product literature.

Kit	Supplier	DNA Isolated	DNA Yield (µg)	Elution Volume (µL)	[DNA] (ng/ µL)
PureLink Mini	Invitrogen	Plasmid	≤30	50	≤600
PureLink Midi			100 to 350	200	500 to 1,750
PureLink Maxi			500 to 850	500	1,000 to 1,700
DNeasy (tissue)	Qiagen	Genomic	25	200	125
DNeasy (cells)			20		100
DNeasy (blood)			5		25

RNA

Yeast RNA standards were prepared as 10 point 1:2 serial dilution series resulting in a concentration range of ~4 to 2,400 ng/µL. Microvolume measurements using both the Epoch spectrophotometer system and NanoDrop were compared to those taken using the BioCell placed in the Take3 multivolume plate and read on the Epoch microplate reader (Figure 3). It is apparent that the linear dynamic range of the Epoch spectrophotometer system is three orders of magnitude, which covers the yield and concentration of most RNA isolation kits (Table 2).

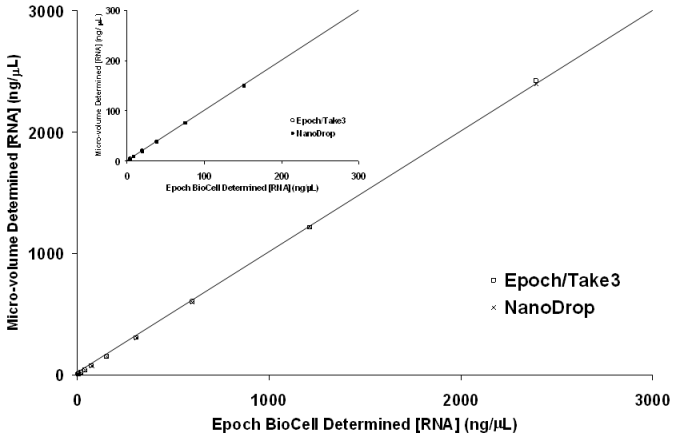

Figure 3. RNA standard curve using dilutions of a purified yeast RNA sample. Abscissa data are considered actual [RNA] as measurements were conducted with 1 cm pathlength using BioCell. Ordinate data are microvolume determinations using an Agilent BioTek Epoch spectrophotometer system and NanoDrop. The straight line is through the origin and has a slope of 1.000, which is considered a perfect equivalence between microvolume and BioCell data. Inset is an exploded view of low RNA concentrations.

Table 2. Expected [RNA] in isolate from some commonly used commercially available DNA isolation kits. RNA yields from tissue samples start with ~25 mg of material; 10^6 HeLa cells were used to generate 20 µg of RNA. Yields are provided as expected results from supplier product literature.

Kit	Supplier	RNA Sample	RNA Yield (µg)	Elution Volume (µL)	[DNA] (ng/ µL)
RNAqueous	Ambion	Liver	100	100	1,000
		Kidney	50	80	625
		Bladder	1.25	50	25
PureLink RNA Mini	Invitrogen	HeLa cells	20	50	400
		Brain	15	50	300

Linear regression analysis was also performed on the RNA standard curve data presented in Figure 3. Both microvolume determinations demonstrated perfect straight line correlations to 4 decimal places (R^2 = 1.0000) and slopes of 0.9900 and 0.9975 for Epoch spectrophotometer system and NanoDrop, respectively. Thus, Epoch spectrophotometer system shows an average accuracy of 1.0% relative to 1 cm pathlength determinations across three orders of magnitude [RNA]; NanoDrop, 0.25%.

Precision

It is evident from Tables 1 and 2 that the concentration of isolated nucleic acid will vary over more than two orders of magnitude depending on the sample type, amount, elution volume chosen, nucleic acid to be isolated, and isolation kit used. The precision of the quantification may differ across such a large dynamic range of concentrations. Figures 4 and 5 demonstrate the precision of replicate measurements of dsDNA from Figure 2 and RNA from Figure 3 respectively, spanning the concentration range depicted in Tables 1 and 2. It is evident that even at low nucleic acid concentration, precision is ≤2.5% CV and averages ≤1.5% CV, suitable for accurate quantification.

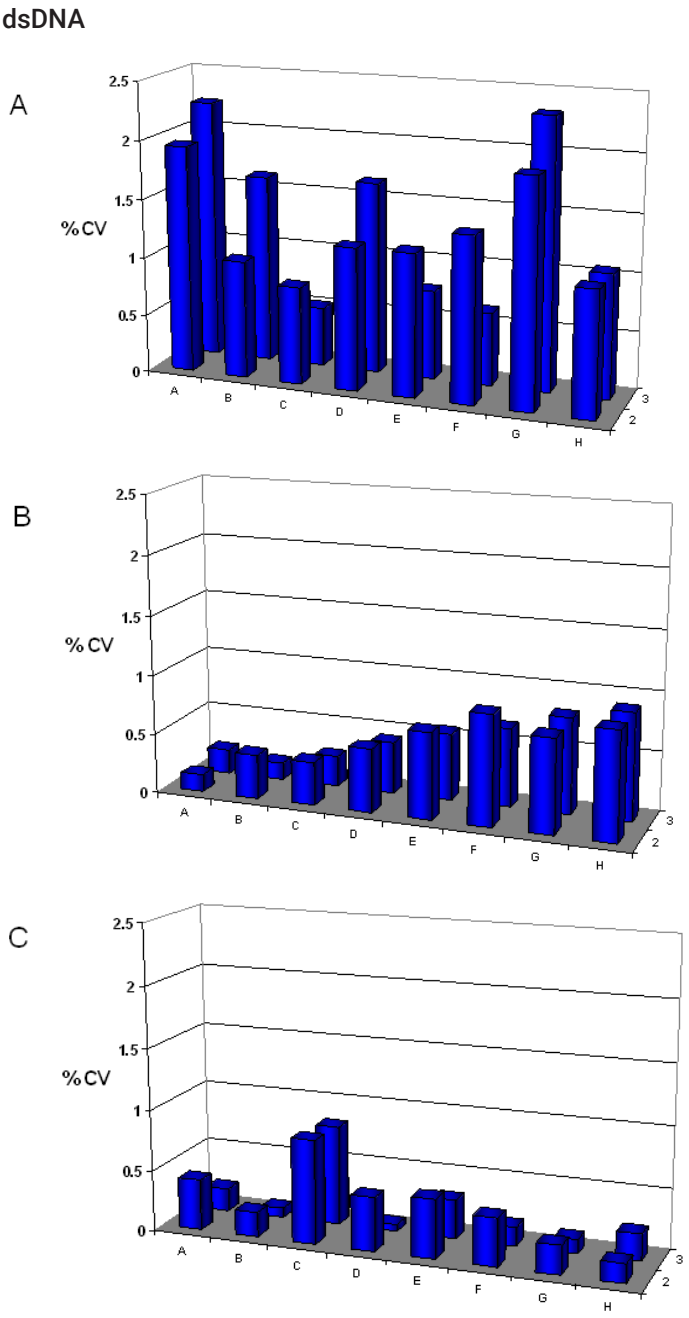

Figure 4. Precision measurements expressed as a %CV from each of the individual 16 microspots at dsDNA concentrations as determined by BioCell. (A) 35.7 ng/µL; (B) 266 ng/µL; (C) 1,050 ng/µL.

RNA

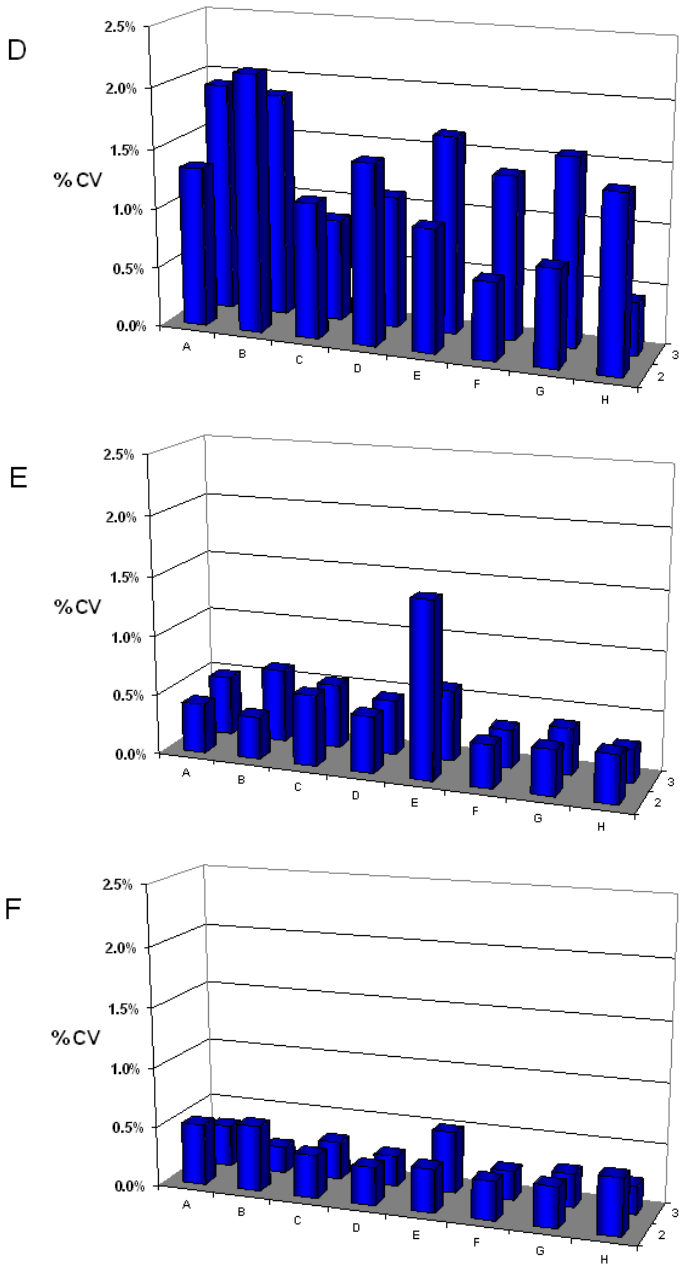

Figure 5. Precision measurements expressed as a %CV from each of the individual 16 microspots at RNA concentrations as determined by BioCell. (D) 38.2 ng/µL; (E) 304 ng/µL; (F) 1,220 ng/µL.

Limit of detection

Table 3 shows the magnitude of the standard deviation for each microspot on the Take 3 plate. All noise signals are below 0.001 OD. The absorbance signal representative of the detection limit can be computed from the average standard deviation from the 16 microspots multiplied by a factor of three. This detection limit is 0.0011 OD.

Table 3. Standard deviation in the blank signal for each of the 16 microspots of the Take3 plate. These data multiplied by a factor of three are representative of an absorbance equivalent to the detection limit for each of the 16 microspots.

	2	3
A	0.00044	0.00039
B	0.00027	0.00026
C	0.00061	0.00020
D	0.00028	0.00029
E	0.00023	0.00038
F	0.00041	0.00047
G	0.00043	0.00037
H	0.00022	0.00060

The limit of detection for both dsDNA and RNA can be computed from the data generated for the linear dynamic range determinations in conjunction with this detection limit expressed as an absorbance signal. The lowest concentration of herring sperm DNA used in the standard curve was determined to be 4.7 ng/µL by using the 1 cm pathlength BioCell; for RNA, it was 4.3 ng/µL. The average background corrected absorbance signal generated by the 16 microspots was determined to be 0.0042 OD for dsDNA; for RNA, it was 0.0053 OD. Therefore, the detection limits for dsDNA and RNA are 1.2 and 0.90 ng/µL, respectively.

Conclusion

The analytical performance of the Agilent BioTek Epoch spectrophotometer system is characterized by a detection limit of 1 ng/µL for the microvolume spectrophotometric determination of nucleic acids at 260 nm. The linear dynamic range for both dsDNA and RNA extends more than three orders of magnitude beyond this to over 2,500 ng/µL. Accuracy relative to 1 cm pathlength determinations with Agilent BioTek BioCells displays a percent difference of ≤2.0% over the full concentration ranges of dsDNA and RNA. Precision over this concentration was typically below 1% CV, but no more than 2.5% CV at low nucleic acid concentrations. This wide concentration range is typical of the yields from current nucleic isolation methods, so good linearity and precision over this range demonstrates broad applicability to nucleic acid quantification. Equivalent performance was seen between microvolume determinations with Epoch spectrophotometer system and NanoDrop.

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