

Compatibility of Synergy™ Multi-Mode Microplate Readers for Micro-Volume Nucleic Acid Quantification

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Nucleic acids are routinely isolated from a wide variety of biological samples for downstream genomic applications. Following isolation, spectrophotometric quantification of the purified nucleic acids is typically performed. Here we demonstrate the compatibility of the Synergy™ Multi-Mode Microplate Reader and Take3™ Multi-Volume Plate for micro-volume analysis using DNA isolated from CHO-M1 cells. Comparative data was derived on Epoch™ and Synergy™ 4 Hybrid Multi-Mode Microplate Readers and shown to have equivalent performance to NanoDrop 2000c.

Introduction

Accurate quantification of isolated nucleic acids is required to ensure that proper amounts of starting material are introduced for maximum efficiency of enzymatic reactions that take place in downstream genomic applications. These applications include sequencing, genotyping, and both differential and quantitative gene expression analysis. Classically, quantification is performed spectrophotometrically at 260 nm in quartz cuvettes with defined measurement pathlengths of 1 cm. Recently, BioTek developed the Epoch Multi-Volume Spectrophotometer System which includes the new Take3 Multi-Volume Plate (see Figure 1). This system enables measurements in 1 cm cuvettes (including BioCell), microplates with variable measurement pathlengths (dependent on plate well geometry and volume of solution) and micro-volume measurements using a defined path length of 0.5 mm^{1,2}. Micro-volume spectrophotometric measurements have the added benefit of requiring no sample dilution prior to analysis.



Figure 1. Take3™ Multi-Volume Plate shown with two BioCells and one standard cuvette in place.

The Take3 Multi-Volume Plate has 16 microspots arranged as 8 rows and 2 columns in standard SBS format for micro-volume analysis. 2 μ L volumes of a sample can be pipetted into individual microwells or a multichannel pipettor may be used to load eight samples simultaneously (see Figure 2).



Figure 2. Loading of the Take3 Multi-Volume Plate performed with an eight-channel pipettor.

Here we show that multi-volume dsDNA determinations can be made using the Take3 Plate and the BioTek Synergy Multi-Mode Microplate Reader.

Comparative data was generated using the Take3 plate with Epoch and Synergy 4 readers and the NanoDrop 2000c.

Materials and Methods

DNA from CHO-M1 cells was isolated to test the applicability of multi-volume analysis on Synergy™ 4 using the Take3 Multi-Volume Microplate. Cells were cultured in T-50 flasks and $\sim 3 \times 10^6$ cells were harvested following trypsin digestion. The cells were collected, $\sim 10^6$ cells per microfuge tube, and washed in MilliQ water twice prior to lysis using 350 μL /tube of RTL buffer in accordance with the AllPrep DNA/RNA/Protein Mini kit protocol (Qiagen). The lysate was then homogenized by passing through a blunt 20-gauge needle at least 5 times, as per the kit protocol. DNA was then purified in accordance with the AllPrep DNA/RNA/Protein Mini Handbook. Elution buffer volume was selected in accordance with estimated recovery quantities; 100 μL EB buffer per column for DNA. Purified samples were measured by UV spectrophotometry by loading the samples in microspot locations on the Take3 2000c, on the pedestal of the NanoDrop and in 1 cm cuvettes. Measurements were performed on the Epoch and Synergy 4 microplate readers (BioTek) and NanoDrop 2000c (Thermo Scientific). 1 μL and 2 μL aliquots of dsDNA were loaded onto the NanoDrop 2000c pedestal and onto the Take3 microspots, respectively. Ten replicate measurements were taken on the NanoDrop 2000c, by individually pipetting 1 μL aliquots to the pedestal, with an absorbance measurement after each. 2 μL of the dsDNA sample was pipetted to each of microspots of the Take3 plate with an 8-channel pipettor, to provide sixteen replicate measurements. All absorbance measurements were recorded. The dsDNA sample was then gravimetrically diluted $\sim 1:20$ or $1:50$ in MilliQ water and read in a low-volume quartz cuvette (Starna Cells) or BioCell (BioTek) in the Take3 plate. Cuvette and BioCell measurements were dilution-factor corrected. The concentration of dsDNA was determined using an extinction coefficient of 50 $\text{ng}/\mu\text{L}/\text{OD}$.

Results

The dsDNA derived from $\sim 3 \times 10^6$ CHO cells was calculated to be at a concentration $\sim 430 \text{ ng}/\mu\text{L}$ (Figure 3). The sample was eluted from the preparative column in a total volume of $\sim 300 \mu\text{L}$ elution buffer for a total yield of approximately 129 μg dsDNA with an A260/280 ratio of ~ 1.9 indicating a highly purified sample preparation. Quantification was very consistent across all instruments and volumes tested, with accuracies consistently within $\pm 2\%$ of the BioCell measurement. Precision in the all the replicate micro-volume measurements was below 2% relative standard deviation.

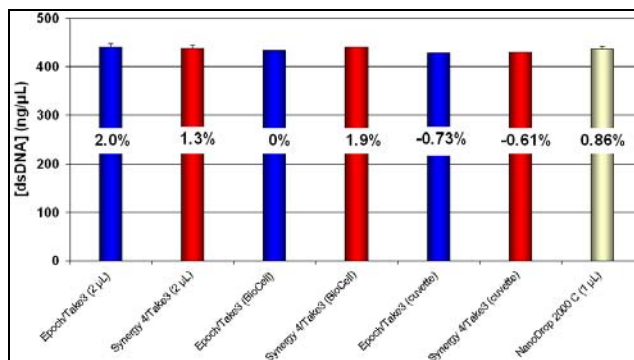


Figure 3. Quantification of CHO cell derived dsDNA on Epoch, Synergy 4, and NanoDrop 2000c. Comparison shows measurements taken using Take3 plate, NanoDrop 2000c, and both BioCell and cuvette with 1 cm pathlength. Inset values indicate percent accuracy compared to BioCell.

Conclusion

The Take3 Multi-Volume Plate allows simultaneous measurement of 16 samples at volumes as low as 2 μL without the need for sample dilution. Additionally, the use of the Take3 plate with either BioCell or standard cuvettes allows measurement of samples at the more conventional 1 cm pathlength or very dilute samples. Here we have shown that measurements using the Synergy 4 Hybrid Multi-Mode Microplate Reader and the Take3 Plate provide precise and accurate nucleic acid quantification when compared to measurements taken at the “gold standard” 1 cm pathlength. The data derived also demonstrates equivalent performance to the dedicated micro-volume spectrophotometer, NanoDrop 2000c.

References

1. Brescia, P. and Banks, P. *Low Volume Nucleic Acid Quantification using a Multi-Volume Spectrophotometer System*, Winooski (VT), BioTek Instruments, Inc., Aug. 2009, Application note.
2. Brescia, P. and Banks, P. *Multi-Volume Analysis of Nucleic Acids Using the Epoch/Take3 Spectrophotometer System*, Winooski, (VT), BioTek Instruments, Inc., Oct. 2009, Application note.