Analytical Performance of a Higher Throughput Multi-Volume Microplate Accessory for Microplate Spectrophotometer Platforms

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Abstract

Accurate determination of molecular concentrations is a prerequisite to the use of purified biomolecules for a multitude of downstream applications. Quantification is routinely accomplished by spectrophotometric analysis. A novel microplate accessory is described here which incorporates the ability to directly measure up to forty-eight low-volume (2µL) samples for direct quantification and two vertical 1 cm pathlength cells, in a standard microplate sized format. Its use allows a researcher to quantify samples without dilution while also allowing more dilute samples to be read in a standard 1 cm pathlength format. The analytical performance of the accessory will be discussed including limit of detection, dynamic range and accuracy for the quantification of total protein and isolated nucleic acids.

Introduction

Current molecular biological and biochemical methods require accurate determination of biomolecular concentrations prior to use. Quantification is routinely accomplished by spectrophotometric analysis at 260 nm for nucleic acids and 280 nm for proteins in a UV transparent vessel. Measurements were historically measured with quartz cuvettes that possessed a fixed path length of 1 cm and are typically associated with high precision and accuracy of measurement. BioTek has recently developed a novel microplate accessory that is capable of accurately measuring up to 48 samples with volumes as low as 2 µL (Figure 1) using the Take3™ Trio Micro-Volume Microplate. The Take3 Trio plate provides a nominal 0.5 mm fixed 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 biomolecular isolation methods. The Take3 Trio plate is also capable of accommodating two BioCells™ extending the range of quantifiable sample concentrations to sub ng/mL by the extension of path length to 1 cm when read with a microplate reader such as the Eon™ Microplate Spectrophotometer (Figure 2).

Here we show the analytical performance of the Take3 Trio for micro-volume analysis including detection limit, linear dynamic range, precision and accuracy for dsDNA, RNA and protein.

Figure 1 – *Take 3 Trio Micro-Volume Microplate. Novel microplate accessory capable of accurately measuring up to 48 samples with volumes as low as 2 µL.*

Figure 2 – *The Eon Microplate Spectrophotometer allows continuous wavelength selection from 200-999 nm, at 1 nm increments. Eon reads 6- to 384-well microplates and is compatible for use with Take3 Trio Micro-Volume plates.*

Material & Methods

Limit of Detection

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 the 48 microspots of the Take3 Trio plate was determined. As with DNA, RNA and protein measurements made in the Linear Dynamic Range determinations outlined below, 260 nm or 280 nm signals were corrected bichromatically at 320 nm. This is standard practice in Gen5™ 2.0 Data Analysis Software and is used by other vendors of instrumentation for micro-volume biomolecular quantification.

Linear Dynamic Range

All double-stranded DNA (dsDNA) and RNA standards were created by serial dilution of a concentrated stock of herring sperm dsDNA or yeast (*Saccharomyces cerevisiae*) RNA, respectively, in TE buffer (10 mm TRIS, 1 mm EDTA, pH=7.0). Bovine serum albumin (BSA) protein standards were created by preparing a 1:2 serial dilution of a stock solution in MilliQ water. Take3 Trio micro-volume data was obtained with undiluted standard samples on an Eon microplate reader. Each standard concentration was loaded as 6-replicates on microspot locations across the Take3 Trio plate using an 8-channel manual pipettor and absorbance read at 260, 280 and 320 nm resulting in 48 sample measurements (Figure 2). BioCell data was acquired using either undiluted or, for higher concentration samples, a 20-fold or 50-fold gravimetric dilution of standard in TE or MilliQ water for either nucleic acids or protein, respectively. All sample measurements were background corrected using a TE buffer blank or MilliQ water, where appropriate, at either 260 or 280 nm for nucleic acids or protein, respectively. All concentrations depicted are based on a 1 cm path length and 50 ng/mL/OD for DNA, 40 ng/mL/OD for RNA and 0.667 µg/mL/OD for BSA.

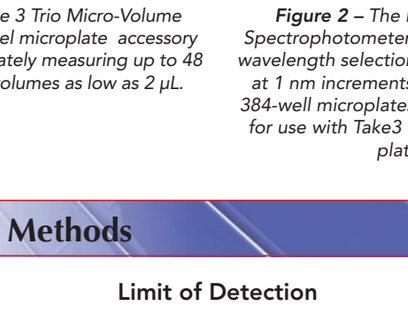

Figure 3 – *Take3 Trio plate map depicting locations of an 8 point serial dilution series of 6 replicates across each row of the accessory plate of either dsDNA, RNA or protein standards.*

HT1080 Cell Biomolecular Purification and Quantification

To test the applicability of the Take3 Trio to provide quantitation of a variety of samples types and concentrations, total DNA, RNA, and protein were isolated from HT 1080 cells from culture. An aliquot of HT 1080 cells containing 4 x 10^6 cells was subject to an 8 point 1:2 serial dilution in buffer and samples prepared using an AllPrep® DNA/RNA/Protein Mini Kit (Qiagen, Valencia, CA, USA) as per the manufacturer's protocol. The 8 point dilution series was added in duplicate onto the Take3 Trio using an 8 channel pipettor. Sample measurements were then performed on an Eon microplate reader controlled by Gen5 2.0 Data Analysis Software (BioTek Instruments, Inc., Winooski, VT, USA). Data analysis was performed using Microsoft® Excel® (Redmond, CA, USA).

Results & Discussion

Limit of Detection

- Calculated limit of detection equals 0.0013 OD from blank measurements

dsDNA

- Average background corrected absorbance signal of 6 microspots was determined to be 0.006 OD
- *Limit of detection for dsDNA is 2.9 ng/µL*

RNA

- Average background corrected absorbance signal of 6 microspots was determined to be 0.004 OD
- *Limit of detection for RNA is 3.2 ng/µL*

Protein

- Average background corrected absorbance signal of 6 microspots was determined to be 0.051 OD
- *Limit of detection for BSA is 70 µg/mL*

Native Nucleic Acid Absorbance at 260 nm

Linear Dynamic Range

dsDNA

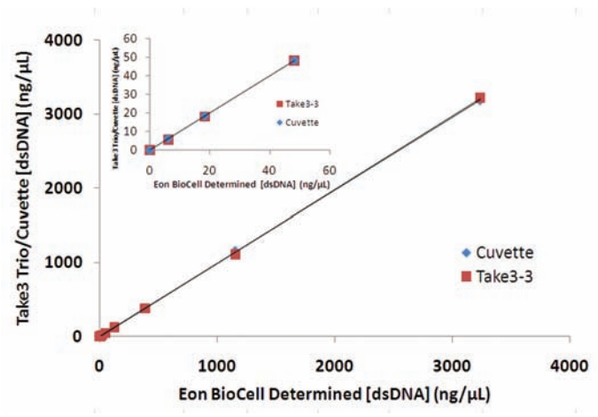

Figure 4 – *dsDNA standard curve using dilutions of a purified herring sperm sample. Abscissa data is considered actual [dsDNA] as measurements were conducted with 1 cm path length using the vertical cell. Ordinate data is micro-volume determinations using the micro-volume plate. The straight line is through the origin and has a slope of 1.000, which is considered a perfect equivalence between micro-volume and vertical cell data. Inset is an exploded view of low dsDNA concentrations.*

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

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.*

- Linear dynamic range spans more than three orders of magnitude
- Covering concentration range from ~ 4.5 ng/µL to ~ 3,250 µg/µL
- Linear regression analysis R2 = 0.999 and slope = 0.993
- Slope is indicative of an average percent difference of <1 % relative to 1 cm pathlength determinations

RNA

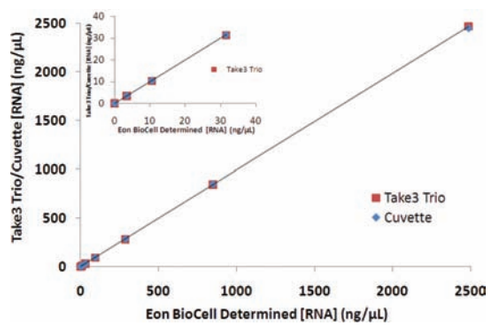

Figure 5 – *RNA standard curve using dilutions of a purified yeast RNA sample. Abscissa data is considered actual [RNA] as measurements were conducted with 1 cm path length using a vertical cell. Ordinate data is micro-volume determinations using micro-volume plate. The straight line is through the origin and has a slope of 1.000, which is considered a perfect equivalence between micro-volume and 1 cm pathlength data. Inset is an exploded view of low RNA concentrations.*

- Linear dynamic range spans three orders of magnitude
- Covering a range from ~ 3.3 to 2,400 ng/µL
- Linear regression analysis R^2 = 1.00) and slope of 0.994
- Slope is indicative of an average percent difference of <1 % relative to 1 cm pathlength determinations

Kit	Supplier	RNA Sample	RNA Yield (µg)	Elution Volume (µL)	[RNA] (ng/µL)
RNAqueous	Ambion	Liver	100	100	1,000
		Kidney	50	80	325
		Bladder	1.25	50	25
PureLink RNA Mini	Invitrogen	HeLa	20	50	400
		Brain	15	50	300

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.*

Results & Discussion *(Continued)*

Precision – dsDNA and RNA

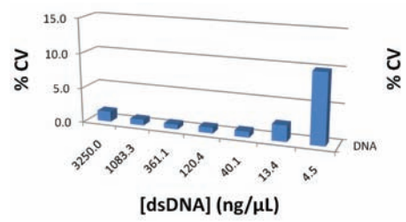

Figure 6 – *Precision of micro-volume methods in the replicate measurements made at each dsDNA standard.*

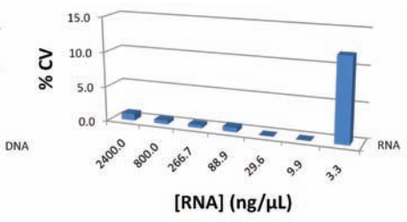

Figure 7 – *Precision of micro-volume methods in the replicate measurements made at each RNA standard.*

- Precision expressed as %CV ≤ 2.3% for [dsDNA] ranging to ~13.4 ng/µL
- Precision expressed as %CV ≤ 1% for [RNA] ranging to ~9.9 ng/µL
- Quantification of nucleic acids is excellent down to low ng/µL concentrations

Protein

Linear Dynamic Range

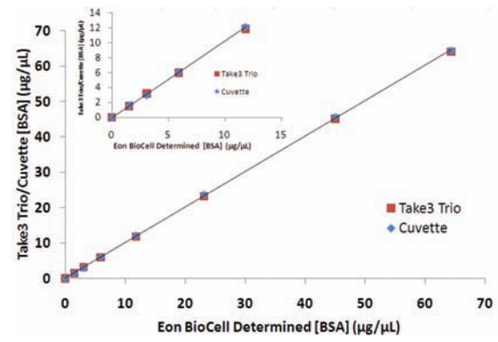

Figure 8 – *Protein standard curve using dilutions of bovine serum albumin measured at 280 nm. Abscissa data is considered actual [protein] as measurements were performed with 1 cm pathlength in a vertical cell. Ordinate data is micro-volume determinations using micro-volume plate. Inset is exploded view of low protein concentration. In both graphs, the line fit represents a slope of 1.003 and demonstrates excellent correlation between micro-volume and 1 cm pathlength determinations.*

Kit	Supplier	Protein Source (*1 X 10^6 cells, **10 mg)	Protein Yield (µg)	Elution Volume (µL)	[Protein] (µg/µL)
AllPrep	Qiagen	NIH/3T3*	50	100	0.5
		HeLa, Jurkat*	60	100	0.6
		Kidney**	1300	100	1.3
		Liver**	1000	100	1.0
		Lung**	800	100	0.8

Table 3 – *Expected protein in isolate when total protein is precipitated using the Qiagen AllPrep kit.*

- Linear dynamic range spans more than three orders of magnitude.
- Covering a concentration range from ~ 0.9 to 60 mg/mL.
- Linear regression analysis $R^2 \geq 0.999$ and slope of 1.003.
- Slope indicates average percent difference of < 1% relative to 1 cm pathlength determinations.

Precision

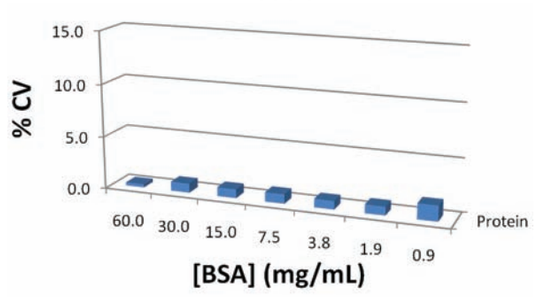

Figure 9 – *Precision of micro-volume methods in the replicate measurements made at each BSA standard.*

- Precision expressed as %CV ≤ 1.5% for [BSA] ranging to ~0.9 mg/mL
- Quantification of protein samples is excellent down to low µg/mL concentrations

Micro-Volume Quantitation of Purified Biomolecules

Cell #	Yield (µg) Genomic DNA	Total RNA	Total Protein
4,000,000.00	13.8	28.7	288.4
2,000,000.00	3.4	22.0	205.5
1,000,000.00	1.5	10.0	169.5
500,000.00	0.7	6.5	41.6

Table 4 – *Calculated yields from native absorbance measurements of purified genomic DNA, total RNA and total protein from the indicated number of HT1080 cells.*

- Values depicted in table 1 correlate well with typical yields as indicated in tables 1-3

Conclusions

1. When using native UV absorbance the micro-volume microplate can provide accurate biomolecular analysis across a broad range of concentrations spanning three orders of magnitude.
2. Detection limits of ~3 ng/µL was observed for nucleic acids at 260 nm and 70 µg/mL when observing purified protein in MilliQ water at 280 nm using the micro-volume accessory plate.
3. Precision was typically below 1% CV, but no more than 2.5% CV at low nucleic acid concentration and ≤ 2% for protein ranging from high BSA concentrations to about 0.9 mg/mL.
4. The Take3 Trio in conjunction with Eon microplate reader provides a system to provide accurate quantification of isolated biological samples from micro-volume, microplate and 1 cm pathlength BioCell or cuvette.

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