The quantification of nucleic acids is a necessary procedure after isolation from samples such as tissues, cells or body fluids. Common applications include PCR, RT-PCR, sequencing restriction digests and ligations. All these applications involve enzymatic reactions where efficiency is dependent on the relative concentrations of nucleic acid, enzyme and other materials, hence the need for quantification. Amounts of RNA and isolated DNA must be quantified before they can be seen as suitable for use in downstream applications such as PCR. Inaccurate quantification of DNA or RNA can lead to a loss of reagents that are designed to be used in high concentration amounts, and an overall loss of time and money in the state of research. Quantification is typically performed using spectrophotometry at 260, 280 and 320 nm. BioTek’s BioCell™ or any standard spectrophotometric cuvette using the Take3™ plate can provide accurate and comparable results. The Take3™ plate has 16 micro-wells allowing for highly accurate determinations with low error.

Materials & Methods

dsDNA Standards

Genomic DNA was prepared from three individual samples of ~1 million mesothelioma cells using the Qiagen's AllPrep DNA/RNA/Protein Mini Kit (Qiagen). Briefly, cells were 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 an 8-channel manual pipettor and absorbances read at 260, 280 and 320 nm. Take3™ micro-volume data was reported as concentration (ng/µL) and standard deviation (µg/µL) from the 16 microspots were used to determine average concentrations and the standard deviation was used as a vertical error bar in Figure 4, below.

DNA from herring sperm dsDNA in TE buffer (10 mM TRIS, 1 mM EDTA, pH=7.0). Take3™ micro-volume data was obtained with undiluted standards. Each standard concentration was loaded twice at multi-microspot location on the Take3™ plate with 16 microspots (2 µL) for micro-volume measurements.

dsDNA from Mesothelioma Cells

An Epoch™ Plate was loaded with two unknown samples of ~1 million mesothelioma cells using an 8-channel manual pipettor. Briefly, cells were homogenized via passage through a syringe fitted with a 20 gauge needle 5-10 times and subject to centrifugation at 15 000 g to remove any residual media and resuspended in 600 µL RTL lysis buffer (supplied with kit). The samples were loaded and read individually in triplicate on the Nanodrop 2000c.

Details of the reproducibility data are provided in Table 1. Table 1 compares the quantification of approximately 10⁶ mesothelioma cells using both BioTek’s Epoch™ / Take3™ and NanoDrop 2000c.

Conclusions

1. Take3™ allows micro-volume quantification of DNA down to 2 µL volumes.
2. Normal path length of 1 cm does not obstruct the need for sample dilution.
3. The provision of 16 micro-wells allows for highly accurate determinations with low error.
4. Take3™ is comparable to gold standard techniques based on a 1 cm path length.
5. Take3™ can be used with standard microplates (96-384 well-diameters) for a myriad of other applications.

References


Table 1. Accuracy vs Nanodrop, Mesothelioma Cells

<table>
<thead>
<tr>
<th>Trial #</th>
<th>Epoch / Take3</th>
<th>Nanodrop 2000c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154 ± 2</td>
<td>151 ± 4</td>
</tr>
<tr>
<td>2</td>
<td>246 ± 2</td>
<td>245 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>105 ± 1</td>
<td>105 ± 2</td>
</tr>
</tbody>
</table>

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, while the latter corresponds to representative of the upper end of the DNA concentration range obtained from the Nanodrop 2000c. The optical densities obtained for these undiluted standards were recorded and measurements taken at 260, 280 and 320 nm respectively for each sample. The data was then compared to the same samples read at 1 cm path length as a control. Both Take3™ plates and Nanodrop 2000c readings were repeated two 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 using an 8-channel manual pipettor and absorbances read at 260, 280 and 320 nm. Take3™ micro-volume data was reported as concentration (ng/µL) and standard deviation (µg/µL) from the 16 microspots were used to determine average concentrations and the standard deviation was used as a vertical error bar in Figure 4, below.

It is apparent from the graph of Figure 4 that there is a 2% difference in absorbance across the broad range of dsDNA concentrations between undiluted micro-volume determinations and 1 cm pathlength determinations made with the BioCell™.