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. 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) per the manufacturer’s protocol. The 8 point dilution series was added in duplicate onto the Take3 Trio using an 8 channel pipette. 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).

Limit of Detection

The limit of detection is defined as the analyte concentration that can provide a signal-to-background ratio of 3:1 for micro-volume determinations and 2:1 for 1 cm pathlength determinations. Results were provided as expected results from supplier product literature.

Linear Dynamic Range

The linear dynamic range spans three orders of magnitude. Covers a concentration range from ~ 0.9 to 60 µg/mL at 280 nm using the Take3 Trio micro-volume accessory plate.

Table 1 – Expected [DNA] in isolate from some commonly used commercially available DNA isolation kits. DNA yields from tissue samples start with 25 µg of material. 10 µL NaOH washes were added to generate 20 µg of RNA. RNA yields are provided as expected results from supplier product literature.

Table 3 – Expected protein in isolate when total protein is precipitated using the Qian Gen kit.

Results & Discussion

• Linear dynamic range spans three orders of magnitude.
• Covering concentration range from ~ 0.9 to 60 µg/mL.
• Linear regression analysis R² ≥ 0.999 and slope of 1.003.

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

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 for protein using the Qian Gen kit.
3. Precision was typically below 1% CV, but no more than 2.5% CV at low nucleic acid concentrations.
4. Protein yields were determined to be accurate and consistent with expected yields from supplier product literature.

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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 nm to 900 nm at 1 nm increments. Read microplate using an 8 well microplate and is compatible for use with Take3 Trio Micro-volume plates.

Table 2 – Expected [RNA] in isolate from some commonly used commercially available RNA isolation kits. RNA yields from tissue samples start with 25 µg of material. 10 µL NaOH washes were added to generate 20 µg of RNA.

Table 5 – Expected protein in isolate when total protein is precipitated using the Qian Gen kit.