

Multi-Volume Analysis of Nucleic Acids Using the Epoch™ Spectrophotometer System

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Nucleic acids isolated from a variety of sample types provide the starting material for a broad range of downstream applications. Quantification of isolated nucleic acid is necessary prior to introduction into these applications. The Epoch Spectrophotometer System provides a means of obtaining accurate measurement over a wide range of sample concentrations and volumes, including micro-volume quantification. Comparison to other micro-volume dedicated devices, such as NanoDrop, shows equivalent performance.

Introduction

The isolation of DNA and RNA from a wide range of samples provides the starting material for a diverse array of applications. Common samples are:

1. tissues of various origins, including human biopsies, animal models, formalin-fixed, paraffin-embedded sections
2. body fluids including blood and buccal swabs
3. eukaryotic and bacterial cells

The isolated nucleic acid can then be used for down stream applications such as those illustrated at the end of the analytical workflows in Figure 1.

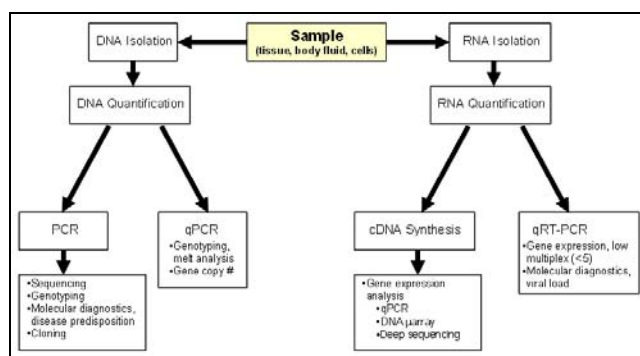


Figure 1. Genomic workflows for downstream applications for DNA and RNA

Essentially, DNA-based applications involve sequencing, genotyping, determining gene copy number and cloning.

For RNA, the goal is gene expression analysis. This can either be done directly from RNA using reverse transcriptase in addition to polymerase in a one step quantitative RT-PCR experiment, or a cDNA library can be made from the isolated mRNA. The advantage of the latter is stability of DNA relative to RNA. This cDNA can then be either used for quantitative PCR for single-plex or low multiplexing of expressed genes, using DNA microarrays for extremely high multiplexing, or even whole transcriptome analysis by deep sequencing methods.

Quantification of both DNA and RNA early in the workflows depicted in Figure 1 is necessary to ensure that relative amounts of nucleic acid template (i.e. the portion of the isolated DNA or RNA that is to be amplified and analyzed) and other reagents, such as enzymes and free nucleotides used for amplification (i.e. in PCR or RT-PCR), are suitable for efficient reactions.

Quantification of Biomolecules by UV Spectroscopy

Standard methods of nucleic acid quantification post-isolation include spectrophotometric absorption and fluorescence when nucleic acids are chelated with a binding dye. The most common method remains absorption spectroscopy due to the peak absorption of DNA and RNA at 260 nm¹. Briefly, a solution of the nucleic acid to be tested is placed inside a vessel and loaded into a spectrophotometer and subject to incident light at a predetermined wavelength and intensity (I₀). The absorption of UV light is measured as it passes through the sample by measuring the intensity (I) at a photo-detector.

The amount of light absorbed is directly related to the concentration of the sample, pathlength and extinction coefficient of the biomolecule of interest. The [Beer-Lambert Law](#) relates the light absorption and concentration of the absorbing molecule via the following equation where $A = \log I_0/I = \epsilon lc$. ϵ refers to the extinction coefficient of the analyte ($\epsilon_{\text{dsDNA}} = 50 \text{ ng-cm } \mu\text{L}^{-1}$; $\epsilon_{\text{RNA}} = 40 \text{ ng-cm}/\mu\text{L}$), l is the pathlength (cm) and c is the concentration of the analyte (ng/ μL), as illustrated in Figure 2.

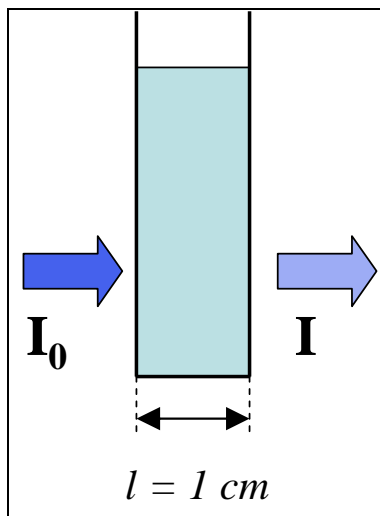


Figure 2. Quantification of biomolecules by UV spectroscopy is dependent on the absorbance of light as it passes through the sample. The relationship is described by *Beer-Lambert Law* $A = \epsilon lc$

Cuvette and Microplate-based Instrumentation

Cuvette-based spectrophotometers are the gold standard instrument for performing spectrophotometric determinations of nucleic acids. Accuracy and precision in the quantification of native nucleic acids at 260 nm using standard 1 cm pathlength cuvettes is unmatched by other instruments and methods. Yet the cuvette-based spectrophotometer does not lend itself to replicate measurements of many samples. After each analysis, the cuvette must be emptied, washed and reloaded with new sample. This is quickly onerous if numerous samples need to be quantified.

To remedy this situation, laboratories have adopted protocols that utilize a 96- or 384-well microplate based format, and suitable microplate instrumentation compatible with UV absorption spectrophotometry. Traditionally, cuvette based measurements are performed in a reader that uses horizontal photometry positioned 90 degrees to the cuvette resulting in a fixed pathlength. Microplate based instrumentation use vertical photometry where the resulting pathlength is due to the height of the sample volume in a microplate well through which the sample is read. The result is a variable pathlength dependent on sample volume and microplate well dimensions (Figure 3). Pathlengths can be corrected for in microplate wells by taking advantage of a small yet significant peak in absorbance for water at 977 nm. Using this phenomenon, the pathlength of any aqueous solution in a microplate well can be determined by comparing the absorbance of that solution at a known pathlength (e.g., 1 cm) to the

absorbance in the microplate well. The ratio of the microplate well absorbance determination to the 1 cm determination is equal to the pathlength in centimeters. The experimental wavelength absorbance divided by the pathlength results in absorbance corrected to 1 cm².

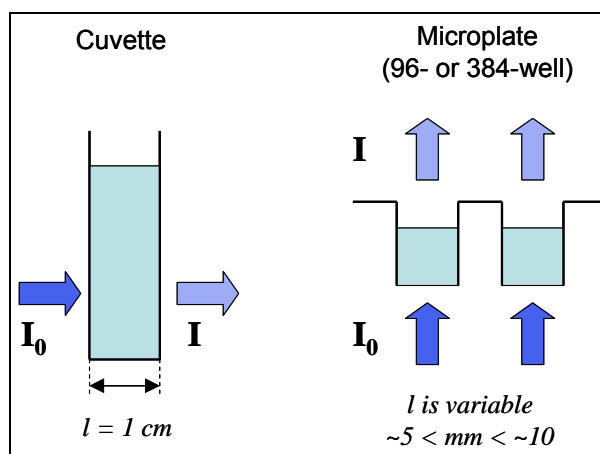


Figure 3. Fixed pathlength using a vertically orientated cuvette ($l = 1 \text{ cm}$) and variable pathlength obtained when using microplates. Pathlength is dependent on sample volume and microplate well dimensions.

Micro-Volume Instrumentation

Micro-volume cuvettes are available which allow analysis of samples in the microliter range with fixed pathlengths a fraction of that available with standard cuvettes. The main advantage of these micro-volume cuvettes is that nucleic acid samples do not have to be diluted to be accurately quantified as the optical density of the measurement is reduced by the same factor as the pathlength reduction. These also have the advantage of being used with conventional cuvette spectrophotometers. Yet the micro-volume cuvettes have the same disadvantage as standard cuvettes when it comes to multiple samples and are often difficult to clean.

A popular device for performing micro-volume quantification of nucleic acids is Thermo Scientific's NanoDrop. There are several models, but the most commonly seen in laboratories is the single channel model. NanoDrop uses a unique sample retention technology to create a consistent, very short pathlength, which allows for analysis without need for dilution. A number of other similar single channel low volume devices are also available on the market.

Epoch™ Spectrophotometer System for Multi-Volume Analysis

The Epoch™ Spectrophotometer System is BioTek's solution for researchers who require low or micro-volume nucleic acid quantification capability. This system includes a Monochromator-based UV-V.s Microplate Reader and Take3™ Multi-Volume Plate. BioTek's Take3 Plate provides a means to perform microvolume quantification on multiple samples, as well as 1 cm measurements with a standard cuvette or the BioCell, a BioTek designed cuvette.

The Take3 plate allows quantification of up to sixteen 2 μL samples with a nominal pathlength of 0.5 mm for micro-volume analysis.

The shorter pathlength allows for direct measurement of highly concentrated samples typically yielded by many low volume preparative kits such as Qiagen's AllPrep DNA/RNA Mini kits.

Additionally, however, a broad range of concentrations and volumes can be measured using the Take3 plate with its ability to accommodate either cuvettes or BioCells on the plate in addition to the microspot locations (Figure 4). The Epoch Microplate Spectrophotometer is used to make the measurements.



A



B

Figure 4. The Take3 plate provides various options to measure samples: A) 16 microspot locations for 2 μL sample loading for micro-volume analysis. B) the plate can accommodate two BioCells and one standard cuvette (each 1 cm pathlength).

Furthermore, the Epoch Microplate Spectrophotometer can measure absorbance of samples in microplates with densities ranging from 6- to 384-wells for a wide range of applications, including ELISAs (Figure 5).

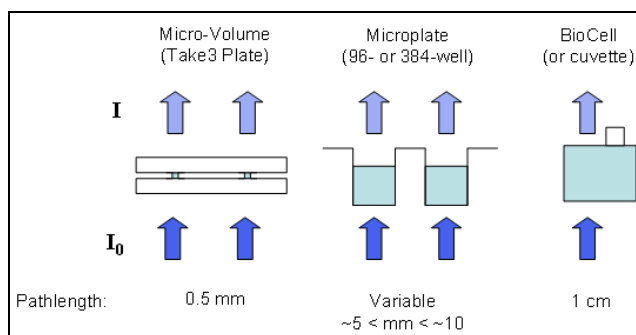


Figure 5. Multi-Volume analysis with Epoch™ Spectrophotometer System

Methods

To test the applicability of the Epoch Spectrophotometer System to provide accurate quantitation of a variety of sample types, volumes and concentrations, total DNA and RNA from Chinese Hamster Ovary (CHO-M1) cells from culture were isolated. Purified samples were then quantified using the Epoch Spectrophotometer System using either undiluted samples in microspot locations on the Take3 plate or after dilution for concentrated samples in a BioCell and 96-well microplates. Additional measurements were performed on the NanoDrop 2000c (Thermo Scientific) for comparison

Results

The dsDNA derived from $\sim 3 \times 10^6$ CHO-M1 cells was determined to be at a concentration of $\sim 440 \text{ ng}/\mu\text{L}$ based on all measurements with different methods/instrumentation (see Figure 8). The sample was eluted from the AllPrep 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 volumes tested, where accuracies fell within 2% of the BioCell measurement (Figure 6).

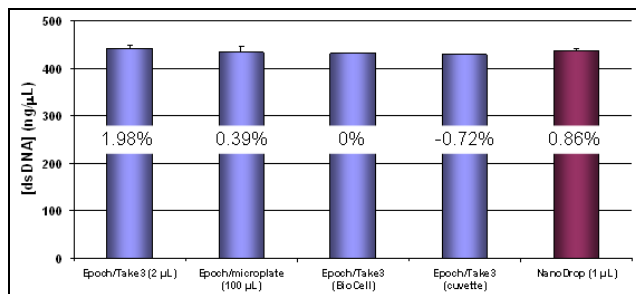


Figure 6. dsDNA quantification from multi-volume capability of Epoch Spectrophotometer System and comparison to NanoDrop. Percentages illustrated on the columns of the bar chart reflect % difference of the measurement relative to a 1 cm pathlength measurement made with BioCell in the Take3 plate.

The RNA yield was approximately 1800 ng/μL in a total volume of 150 μL for a total of approximately 270 μg RNA with an A260/280 ratio of ~2.0, again indicative of good quality sample preparation (Figure 10). Quantification was again found to be consistent across all volumes tested, where accuracies fell within ~ ± 2.5% with the largest deviations derived from measurements taken in the microplate format (Figure 7).

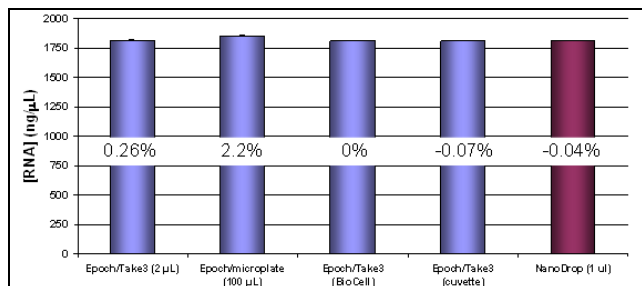


Figure 7. RNA quantification from multi-volume capability of Epoch Spectrophotometer System and comparison to NanoDrop. Percentages illustrated on the columns of the bar chart reflect % difference of the measurement relative to a 1 cm pathlength measurement made with BioCell in the Take3 plate.

Conclusion

Quantification of nucleic acids after isolation from biological samples is a standard procedure for many applications. The Epoch Microplate Spectrophotometer, used with the Take3 plate, makes a system that can provide accurate quantification from micro-volume, microplate and 1 cm pathlength cuvettes and BioCell. Micro-volume quantification using the microdots of the Take3 plate obviates the need to dilute the isolated nucleic acid samples. Up to 16 samples can be quantified at the same time with the Take3 plate. Comparisons to 1 cm pathlength measurements and to NanoDrop demonstrate an equivalence of analytical performance.

References

1. Sambrook and Russell. Molecular Cloning: A Laboratory Manual (3rd Ed.). Cold Spring Harbor Laboratory Press. 2001.
2. Held. "Nucleic Acid & Protein in the Microplate Format" CHIMIA 2001, vol.55, issue 1-2, p. 40-42.