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The NanoDrop Eight Spectrophotometer detects contaminating nucleic acids in mammalian DNA and RNA preparations

Introduction

Understanding nucleic acid sample quality and quantity is integral for many life science applications, reducing the occurrence of costly delays caused by troubleshooting downstream experimental failures. The Thermo Scientific™ NanoDrop™ Eight Microvolume UV-Vis Spectrophotometer measures eight samples at a time and provides you the ability to measure the concentration of biomolecules for high-throughput assays using a 1–2 µL sample size without the need for dilutions. With a measurement time of less than 20 seconds, you can easily insert the NanoDrop Eight Spectrophotometer into your high-throughput workflows.

The Thermo Scientific Acclaro™ Sample Intelligence Technology integrated within the NanoDrop Eight Spectrophotometer's software utilizes chemometrics to detect RNA in dsDNA sample preparations and dsDNA in RNA preparations to then calculate a corrected dsDNA or RNA concentration, respectively. Historically, the A260/A280 purity ratio has been utilized to assess nucleic acid sample purity; however, nucleic acid contaminants at low concentrations, such as RNA contamination in dsDNA samples, have a negligible effect on the purity ratio, and the contaminant identity is not easily determined by a change in the A260/A280 purity ratio or by visualizing the UV-Vis spectrum. Acclaro Technology's contaminant analysis capability eliminates the need for purity ratio assumptions and reports the contaminant present, contaminant absorbance, and a corrected sample concentration (Figure 1).

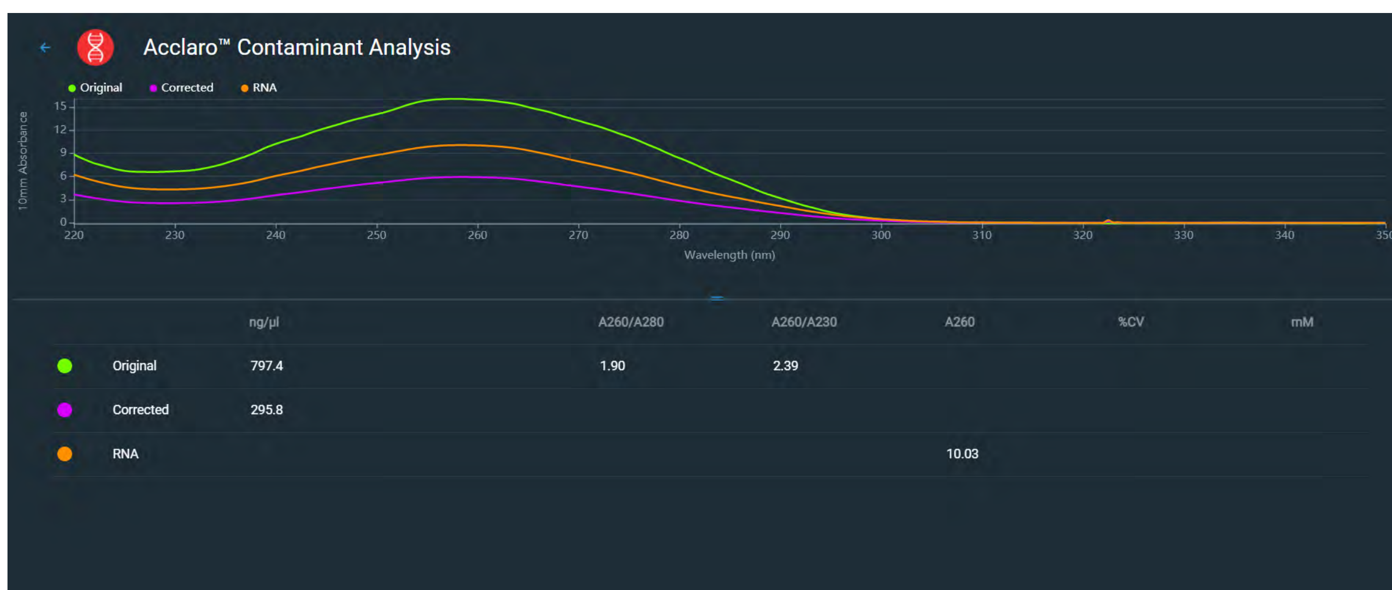

Figure 1: Acclaro Technology's contaminant analysis screen outlining the original concentration, corrected dsDNA concentration, and the absorbance contribution of RNA contamination. The original spectrum is shown in green, the corrected spectrum in pink, and the contaminating RNA spectrum in orange.

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Materials and methods

Total RNA and genomic DNA from mouse tissue (BioChain Institute Inc., R1334035-50 and D1334999-G01) and RNA and genomic DNA from the MCF-7 cell line (BioChain Institute Inc., R1255830-50 and D1255830) were dialyzed and diluted in tris-EDTA buffer (TE pH 8.0, Fisher Scientific, BP2473500) and made into various DNA/RNA mixtures according to percentage of absorbance contribution. Triplicates of each mixture were measured on the NanoDrop Eight Spectrophotometer using fresh 1.0 µL aliquots per replicate for the dsDNA and RNA applications.

The NanoDrop Eight Spectrophotometer's Acclaro Technology-corrected results from the mouse and MCF-7 DNA/RNA mixtures were compared with the theoretical concentration and the original, uncorrected concentration in Figures 2 and 3 using the dsDNA and RNA applications, respectively. Acclaro Technology calculated an original, uncorrected concentration and a corrected concentration based on a modified Beer's Law equation and the absorbance contribution at 260 nm.

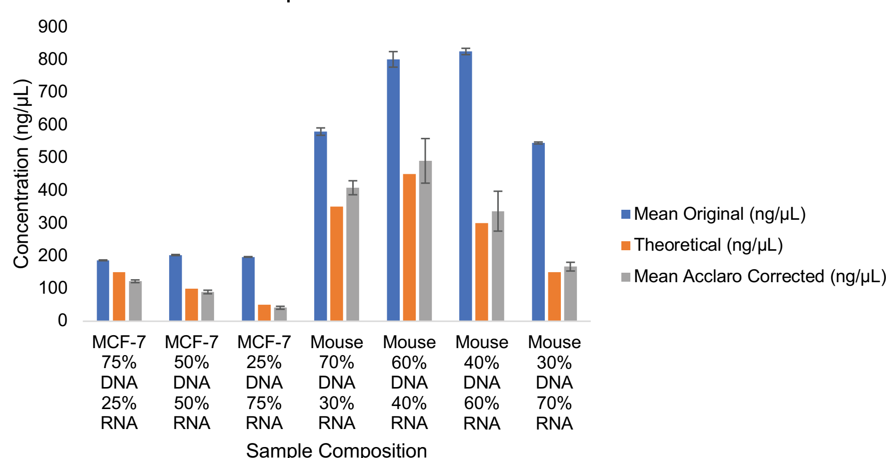

Figure 2: Comparison of the concentration reported by the Acclaro Technology for different sample compositions of DNA and RNA based on percentage of absorbance contribution. DNA and RNA from either the MCF-7 cell line or mouse tissue were mixed according to absorbance percentage and were measured using the dsDNA application. The mean original concentration (blue bars), the theoretical concentration (orange bars), and the mean Acclaro Technology software-corrected concentration (gray bars) were reported by the NanoDrop Eight Spectrophotometer's software. Error bars represent the standard deviation.

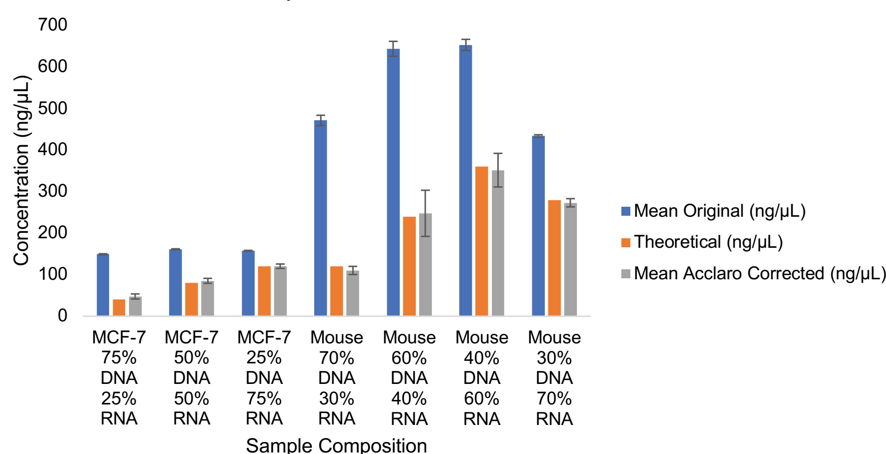

Figure 3: Comparison of the concentration reported by the Acclaro Technology for different sample compositions of DNA and RNA based on percentage of absorbance contribution. DNA and RNA from either the MCF-7 cell line or mouse tissue were mixed according to absorbance percentage and were measured using the RNA application. The mean original concentration (blue bars), the theoretical concentration (orange bars), and the mean Acclaro Technology software-corrected concentration (gray bars) were reported by the NanoDrop Eight Spectrophotometer's software. Error bars represent the standard deviation.

Results

In Figures 2 and 3, the Acclaro Technology's software-corrected mean concentration from the NanoDrop Eight Spectrophotometer was graphed against the original, uncorrected concentration and the theoretical concentration for the mouse and MCF-7 DNA/RNA mixtures with standard deviation shown as error bars. Since nucleic acids absorb at 260 nm, the original, uncorrected concentration is inflated compared to the Acclaro Technology's software-corrected concentration when DNA and RNA are both contributing to absorbance.

With the inclusion of the Acclaro Technology in the NanoDrop Eight Spectrophotometer's software, the corrected nucleic acid concentration was calculated after correcting for the contaminant absorbance contribution. This feature allows for simultaneous nucleic acid purity and quantity assessments. All the Acclaro Technology's software-corrected concentrations fall within ±20% of the theoretical concentration, with most samples within ±10%.

Conclusion

Contaminating nucleic acids in dsDNA or RNA preparations can cause costly delays in applications such as qPCR, where exact quantitation is crucial for a successful experiment. Since RNA and dsDNA both absorb at 260 nm, the true nucleic acid concentration will be overestimated with a copurified contaminant present. This overestimation can lead to experimental failures and require extensive troubleshooting. The ease with which the Acclaro Technology corrects for contaminating nucleic acids will save time, effort, and associated costs by improving sample purity and quantity assessments.

The function of the Acclaro Technology makes the nucleic acid purity assessment clear and simple. With each measurement of a nucleic acid sample, the NanoDrop Eight Spectrophotometer takes quality assessment a step further by outlining the contaminant identification, absorbance contribution, and the corrected sample concentration. The results from the experiments above indicate the NanoDrop Eight Spectrophotometer, which includes the Acclaro Technology in its software, can be implemented into many molecular biology workflows to obtain an accurate and advanced nucleic acid evaluation for downstream success.

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