

Detection of RNA contamination in Mammalian DNA preparations

Using advanced UV-Vis algorithms to improve specificity

Introduction

Quantification of nucleic acids has traditionally been performed by determining the UV absorbance at 3 analytical wavelengths: 230 nm, 260 nm, and 280 nm. The information derived from these absorbance measurements allows scientists to measure nucleic acid concentration and to have an indication of sample purity. A disadvantage of this approach is the lack of specificity. Thus, any contaminant that absorbs at these wavelengths will lead to inaccuracies in a nucleic acid concentration result.

The Thermo Scientific™ NanoDrop™ One/One^C Spectrophotometer features Thermo Scientific™ Acclaro™ Intelligence Technology Software which uses full-spectrum data and advanced algorithms to identify common nucleic acid contaminants and provide corrected nucleic acid concentrations. In this technical note, we show how a newly introduced feature in Acclaro software allows users to detect and correct for RNA contamination in DNA preparations or DNA contamination in RNA preparations.

Materials and methods

DNA and RNA were prepared from HeLa cells, treated with RNase or DNase to remove contaminant nucleic acid, and dialyzed in TE buffer, pH 8.0. Initial stock concentrations were determined on the NanoDrop One spectrophotometer against a TE blank. Various mixtures of DNA and RNA were made (as shown in Figure 1), and triplicates of each sample were read on the instrument. A fresh 2.0 μL aliquot of the appropriate mixture was used for each replicate. The following DNA concentration results were calculated:

- Theoretical DNA concentration: baseline corrected $A_{260} * 50 \text{ ng}/\mu\text{L} * (\% \text{DNA}/100)$.
- Uncorrected DNA concentration: baseline corrected $A_{260} * 50 \text{ ng}/\mu\text{L}$.
- Acclaro-corrected DNA concentration: Acclaro-corrected $A_{260} * 50 \text{ ng}/\mu\text{L}$.

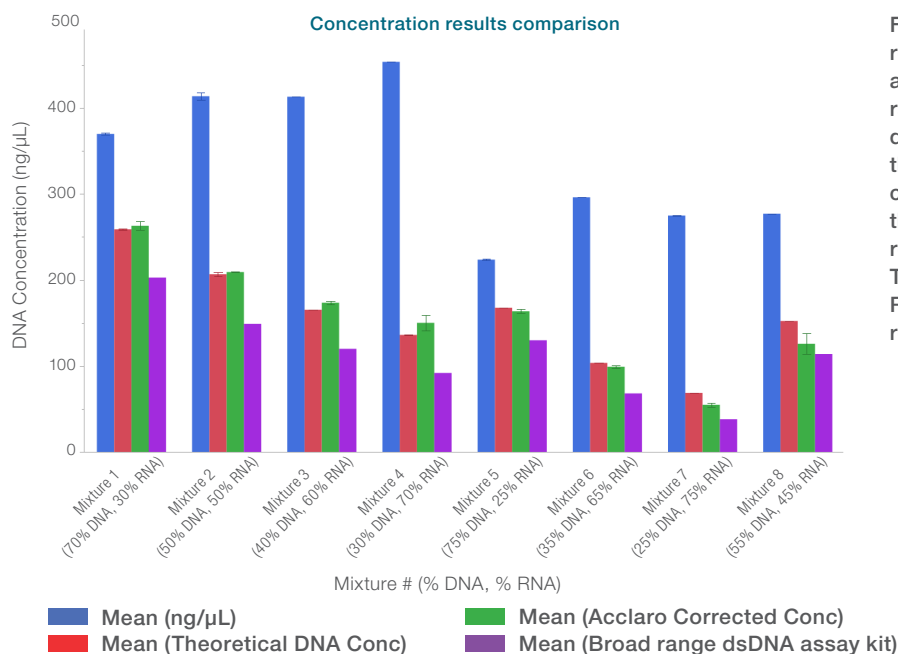


Figure 1: The bar graph compares the concentration results of various mixtures of DNA and RNA using different approaches. The purple bars represent data using a broad range dsDNA assay kit. This assay uses a fluorescence dye, that specifically binds DNA. The blue bars represent the data when a direct A260 absorbance is used to calculate the DNA concentration. The red bars represent the theoretical target DNA concentrations. The green bars represent the Acclaro-corrected DNA concentrations. The DNA and RNA preparations used for the DNA/RNA mixtures were isolated from HeLa cells. Error bars represent the standard deviation from the mean.

The theoretical, uncorrected, and Acclaro-corrected DNA concentrations were compared to those obtained with a DNA-specific fluorescent assay. Fluorescence assays were performed using a broad range dsDNA assay kit and a fluorescence instrument. A standard curve was generated according to the assay kit's protocol. For this assay, standards were prepared by adding 10 μL of the appropriate DNA standard to 200 μL of working solution. Nucleic acid samples were then prepared by adding 2.0 μL of sample to 200 μL of working solution. DNA standards and samples were incubated at room temperature for 5 minutes and then read on the fluorescence instrument.

Mean DNA concentrations and standard deviations for each sample are shown in Figure 1.

Results

When calculating DNA concentration in the presence of contaminating RNA, the highest degree of inaccuracy was observed when uncorrected A260 absorbance was used to calculate the DNA concentration. Both macromolecules (i.e., RNA and DNA) absorb light at 260 nm; therefore, using absorbance at 260 nm to calculate the concentration of DNA can lead to an overestimation of the actual concentration of DNA in a sample.

The Acclaro-corrected results (green bars) demonstrate that greater accuracy is achieved in the determination of DNA concentration when using the Acclaro feature. The Acclaro algorithm uses the full spectral data in conjunction with multivariate mathematics to specifically determine the concentration of DNA in a DNA/RNA mixture. The DNA concentrations obtained after Acclaro correction were much closer to the those obtained by using a DNA-specific fluorescence dye.



NanoDrop One Spectrophotomer

Conclusion

RNA contamination in genomic DNA preparations is a common issue in molecular biology workflows. When measuring samples with traditional spectrometry, copurified RNA will artificially inflate the concentration of DNA. Although using specific DNA-binding dyes may provide a more accurate determination of DNA concentration than absorbance at 260 nm, contamination of a DNA sample with copurified RNA can have adverse effects in modern genomic workflows.

We demonstrated that by using full spectral data and multivariate mathematical algorithms, researchers can overcome the disadvantages of both type of assays mentioned. The Acclaro software that runs the Nanodrop One spectrophotometer allows the identification of RNA contamination in a DNA sample and provides a corrected concentration result. These two factors will allow molecular biologists to quickly troubleshoot difficult extractions and improve downstream results.

For more information about resolving nucleic acid contamination with NanoDrop One systems visit thermofisher.com/NanoDropQuant