



Using the NanoDrop One/One^C Spectrophotometer to determine DNA/RNA contamination in nucleic acids for RT-qPCR/qPCR quality control

Abstract

The Thermo Scientific™ NanoDrop™ One/One^C Microvolume UV-Vis Spectrophotometer can be seamlessly implemented into the qPCR/RT-qPCR workflow for nucleic acid quality control. When conducting a PCR experiment, the nucleic acid template must be added in a specific concentration and be free of contaminating materials. The Thermo Scientific™ Acclaro™ Sample Intelligence Technology integrated into the NanoDrop One/One^C instrument has the capability of quantifying RNA or DNA contamination present in a DNA or RNA sample, respectively. In this study, the effects on quantitative PCR and reverse transcription-quantitative PCR (qPCR and RT-qPCR) results were investigated after spiking nucleic acid samples with RNA or DNA, targeting the BRCA1 gene. The results indicate nucleic acid contamination increases the quantification cycle (C_q) and inflates the absorbance and concentration values. The NanoDrop One/One^C instrument, along with the Acclaro technology capabilities, ensures nucleic acids are pure and at an accurate concentration for qPCR and RT-qPCR, saving time and resources by preventing failed reactions.

Introduction

According to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, it is crucial to maintain quality control of nucleic acid samples; otherwise, variability in PCR results will be demonstrated.¹ Sample quality control includes maintaining RNA preparations that are free of DNA and DNA preparations that are free of RNA. The MIQE guidelines suggest using the A260/A280 purity ratio as a method of determining the purity of the extracted nucleic acid sample.¹ Historically, purity ratios have been used to determine the purity of DNA and RNA samples with an A260/A280 purity ratio of ~1.8 for pure DNA and ~2.0 for pure RNA prior to setting up a qPCR or RT-qPCR assay. However, traditional spectrophotometers cannot determine contaminants that co-absorb with the target sample at the 260 nm analytical wavelength, such as DNA contamination in an RNA sample. This shortcoming leaves the experimenter with an overestimated concentration and contaminated nucleic acid sample, causing an inaccurate qPCR or RT-qPCR result.²

Advantage of NanoDrop One Spectrophotometer in RT-qPCR and qPCR workflows

To help ensure nucleic acid samples are free of contaminants that affect qPCR/RT-qPCR results, the Acclaro technology identifies common contaminants in nucleic acid preparations and reports a corrected sample concentration (Figure 1). The Acclaro technology is integrated into the NanoDrop One/One^c instrument software.

Figure 2 outlines the contaminants that are identified by the Acclaro technology for the dsDNA and RNA applications. The software will report the original concentration without any Acclaro algorithm correction applied, a corrected sample concentration, and the contaminant's absorbance contribution to the analytical wavelength.

In addition to the Acclaro technology features, the NanoDrop One/One^c Spectrophotometer utilizes microvolume sample measurements, conserving the extracted nucleic acid product for downstream testing. In less than 8 seconds, the NanoDrop One/One^c instrument reports the A260/A280 and A260/A230 purity ratios along with the sample concentration without the need for dilutions, providing helpful details prior to the PCR reaction.

Materials and method

- For RT-qPCR, total RNA from human lymphocytes (isolated by BioChain, R1254148-1) and genomic DNA (isolated by BioChain, D1234999-G01) were prepared by dialyzing and diluting in Tris-EDTA (TE) buffer (Fisher BioReagents, pH 7.6, BP2474500). 100 ng/µL RNA samples were spiked with 25 ng/µL of genomic DNA. BRCA1 was amplified using TaqMan™ RNA-to-CT™ 1-Step Kit (Applied Biosystems, 4392653) and the BRCA1 TaqMan® Gene Expression Assay (assay ID Hs01556193_m1).
- For qPCR, genomic DNA from human tumor cells (isolated by BioChain, D1255811) and total RNA from human lymphocytes (isolated by BioChain, R1254148-1) were prepared by dialyzing and diluting in Tris-EDTA (TE) buffer (Fisher BioReagents, pH 7.6, BP2474500). 100 ng/µL DNA samples were spiked with 50 ng/µL of RNA. BRCA1 was amplified using the PrimeTime™ Gene Expression Master Mix for qPCR (Integrated DNA Technologies, Inc., 1055770), and BRCA1 primers and a probe were designed by the Integrated DNA Technologies, Inc. PrimerQuest™ Tool.
- Spiked RNA and gDNA samples were measured on the NanoDrop One/One^c instrument to determine the corrected and original concentrations reported by the Acclaro technology (Figure 3). The original and corrected concentrations were subsequently diluted to 25 ng/µL prior to loading on the qPCR machine.
- RT-qPCR and qPCR were conducted on the QuantStudio™ 6 Pro Real-Time PCR System (Applied Biosystems, A43159).



Figure 1: RNA spectra with DNA contamination, identified by Acclaro technology. The NanoDrop One/One^c instrument software reports the corrected RNA concentration and spectrum in yellow.

dsDNA	RNA
Protein	Protein
Phenol	Phenol
Guanidine HCl	Guanidine isothiocyanate
RNA	DNA

Figure 2: Contaminants identified by the NanoDrop One/One^c instrument's Acclaro technology for the RNA and dsDNA applications.

Sample	Contaminant concentration	NanoDrop original concentration (ng/µL)	NanoDrop corrected concentration (ng/µL)
Control	N/A	101.7	N/A
dsDNA	50 ng/µL RNA	102.8	46.0
RNA	25 ng/µL dsDNA	104.5	81.5

Figure 3: The concentrations of nucleic acid samples spiked with contaminants were measured on the NanoDrop One/One^c Spectrophotometer to trigger Acclaro Contaminant ID analysis using the RNA and dsDNA applications, depending on the sample type.

DNA contamination in RNA preparations

Genomic DNA (gDNA) contamination is a significant concern for RNA samples as gDNA amplification can occur alongside cDNA during PCR, leading to inaccurate quantification after reverse transcription.² With traditional spectrophotometry, dsDNA and RNA cannot be distinguished from each other as they co-absorb at 260 nm, inflating the output concentration. The inflated concentration causes the experimenter to over-dilute their RNA for RT-qPCR. With an RNA concentration that is too dilute for RT-qPCR, one can expect high variability between replicates due to stochastic effects or inhibition of amplification.³ Figure 4 shows the effect on C_q when RNA was spiked with gDNA. The C_q for both the corrected and original concentrations were elevated when compared to the control. The C_q of the corrected concentration remains higher than the control because the gDNA contamination was still present in the sample. The Acclaro technology serves as a useful tool for determining DNA contamination in a sample of RNA, but the results suggest the RNA must be purified prior to qPCR to improve accuracy.

To mitigate the effect on C_q due to gDNA contamination, Bustin (2002) recommends a DNase treatment be performed prior to RT-qPCR. After the DNase treatment is completed, the user can remeasure their sample on the NanoDrop One/One^C instrument to determine the purity and concentration of their RNA sample before downstream application.

RNA contamination in DNA preparations

Extracted DNA samples are regularly contaminated with RNA at a rate of 28-52% depending on the tissue type per Sanchez et al. (2015), resulting in an overestimation of the DNA concentration when measured with UV-Vis spectrophotometers. The effect of RNA contamination on qPCR induces variability in results as qPCR requires the DNA starting amount to be standardized across replicate wells, creating challenges when DNA concentration is overestimated in the presence of contaminants.⁴ Figure 5 describes the effect on C_q when gDNA was spiked with RNA, where the C_q was elevated for the original concentration and returned to normal when the corrected concentration was used. The results indicate the corrected DNA concentration provided by the Acclaro technology restores the C_q to the control. However, the recommendation remains to purify the DNA prior to qPCR to ensure the most accurate and reproducible results.

Sanchez et al. (2015) suggests an RNase step prior to qPCR to eliminate contaminating RNA in the DNA sample. The purity and concentration of the dsDNA can be measured on the NanoDrop One/One^C Spectrophotometer for accurate quantification prior to qPCR.

Comparing the NanoDrop corrected vs. original concentrations and the effect on C_q for DNA contamination

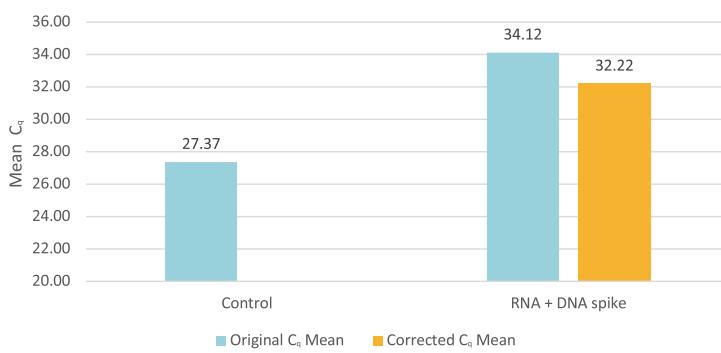


Figure 4: The original sample was diluted to 25 ng/ μ L from the original concentration reported by the NanoDrop One/One^C Acclaro technology and the corrected concentration was used for diluting the corrected sample to 25 ng/ μ L.

Comparing the NanoDrop corrected vs. original concentrations and the effect on C_q for RNA contamination

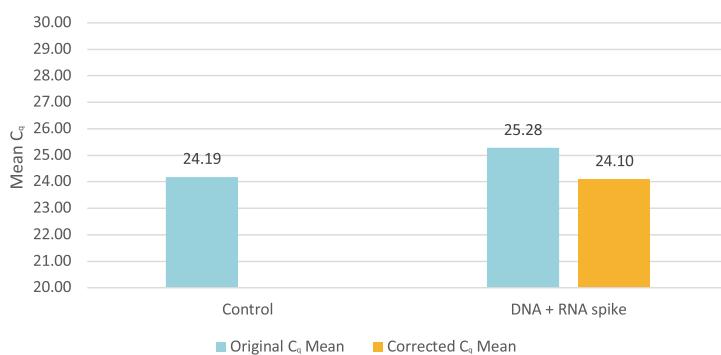


Figure 5: The original sample was diluted to 25 ng/ μ L from the original concentration reported by the NanoDrop One/One^C Acclaro technology and the corrected concentration was used for diluting the corrected sample to 25 ng/ μ L.

Conclusion

Variability in qPCR or RT-qPCR results due to contaminated nucleic acid samples can have negative effects for those using PCR in clinical diagnostics or genetic testing where accurate quantification is crucial. The NanoDrop One/One^C Spectrophotometer can be seamlessly implemented in the RT-qPCR or qPCR workflow to ensure nucleic acid samples are free of contaminating DNA or RNA, respectively. Traditional spectrophotometers are not able to distinguish RNA from dsDNA and vice versa as both nucleic acids absorb at 260 nm. With the Acclaro technology integrated in the NanoDrop One/One^C instrument, the user can obtain a quantified concentration of the target nucleic acid and the contaminating nucleic acid, saving valuable time and resources for downstream quantitative PCR.

References

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