

Myth Busted: A NanoDrop ND-1000 Spectrophotometric Reading Is Insufficient to Assess RNA Quality

Introduction

According to the recently published quantitative real-time PCR experiment publication guidelines, or MIQE (Bustin et al. 2009; <http://www.rdml.org/miqe.php>), providing RNA integrity data is essential when publishing real-time quantitative PCR (qPCR) and reverse transcription qPCR (RT-qPCR) data. RNA integrity is critical in qPCR and RT-qPCR experiments in order to obtain gene expression results that are reliable and reproducible, and therefore publishable.

The Experion™ automated electrophoresis system (Bio-Rad Laboratories, Inc.) provides an automatic assessment of RNA integrity by providing the RNA quality indicator (RQI) in addition to the electropherogram, gel view, and 28S/18S ratio and concentration (Figure 1, Table 1). Here we illustrate that the NanoDrop (ND-1000) spectrophotometer (Thermo Fisher Scientific, Inc.) can assess concentration and also provide some purity data (via $A_{260/280}$ nm and $A_{260/230}$ nm readings) for the same mouse liver total RNA sample that has been degraded to varying degrees (Table 1). However, the ND-1000 spectrophotometer does not provide RNA integrity data.

When mouse liver samples (with varying degrees of integrity, as generated by heating samples at 90°C) were analyzed on both the Experion and ND-1000 systems, Experion system results show:

- Samples 1, 2, and 3 (RQI 9.8, 9.2, and 8.1, respectively) are highly intact and can be used in downstream applications with confidence
- Samples 4 and 5 (RQI 6.5 and 5.9, respectively) are somewhat degraded and may or may not be usable depending on the application
- Samples 6, 7, and 8 are highly degraded (RQI 2.2, 2.0, and 1.8, respectively) and should not be used for downstream applications (Figure 2)

ND-1000 spectrophotometer results indicate that all samples are good quality.

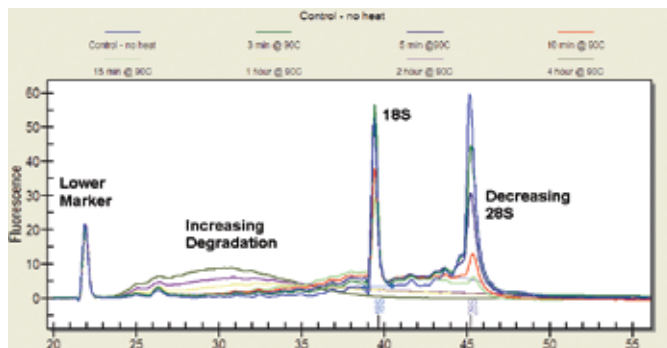


Fig. 1. Experion system electropherogram overlay of mouse liver total RNA sample progressively degraded with heat. With longer heat exposure, there is an observed decrease in the 28S and 18S peaks with degradation peaks appearing between the lower marker and the 18S peak.

Table 1. Experion system and ND-1000 spectrophotometer assessment of the same mouse liver total RNA sample. Experion system results give fast (11–12 samples in 30 min) and the most complete information (electropherogram and virtual gel profile, RQI value, and 28S/18S ratio) to help determine RNA integrity. (■), very little or no degradation; (■), some degradation; (■), significant degradation.

Sample Number	Sample Name	Experion Automated Electrophoresis System			NanoDrop ND-1000 Spectrophotometer		
		Ratio 28S/18S	RQI	RQI Class	Conc, ng/μl	$A_{260/280}$ *	$A_{260/230}$ *
1	Control — no heat	1.60	9.8	■	115	1.90	2.44
2	3 min @ 90°C	1.23	9.2	■	114	1.93	2.40
3	5 min @ 90°C	0.89	8.1	■	115	2.06	2.37
4	10 min @ 90°C	0.50	6.5	■	115	2.03	2.37
5	15 min @ 90°C	0.15	5.9	■	116	2.02	2.31
6	1.0 hr @ 90°C	0.46	2.2	■	109	1.99	2.18
7	2.0 hr @ 90°C	0.81	2.0	■	117	2.00	2.32
8	4.0 hr @ 90°C	0.00	1.8	■	118	1.89	2.23

* Note: Generally accepted ratios ($A_{260/280}$ and $A_{260/230}$) for good quality RNA are >1.8; the ND-1000 spectrophotometric readings indicate that all samples are good quality.

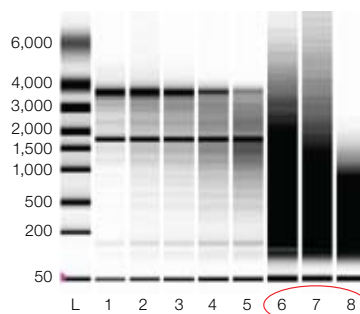


Fig. 2. Experion system virtual gel clearly shows that samples 6, 7, and 8 are highly degraded.

Conclusions

NanoDrop ND-1000 spectrophotometric readings provide only part of the information needed for reliable RT-qPCR results. Conversely, the Experion system provides a complete evaluation of total RNA, with all results automatically generated, saved, and easily exported.

Reference

Bustin SA et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 55:4, 611-622.

NanoDrop is a trademark of Thermo Fisher Scientific, Inc.



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