TECHNICAL NOTE

No. TN53457

NanoDrop Eight system identifies protein and phenol in DNA samples

Acclaro Sample Intelligence Technology expands NanoDrop Eight Spectrophotometer capability

Abstract

This technical note reviews the performance of the Thermo Scientific™ Acclaro™ Sample Intelligence Technology integrated within the Thermo Scientific™ NanoDrop™ Eight Spectrophotometer Software. The Acclaro Software utilizes chemometric principles to detect the amount of protein, phenol, guanidine HCl, and/or RNA in dsDNA sample preparations and calculates a corrected dsDNA concentration. The accuracy and reproducibility of the Acclaro Software in determining phenol and protein contaminants in dsDNA samples and calculating a corrected concentration were analyzed.

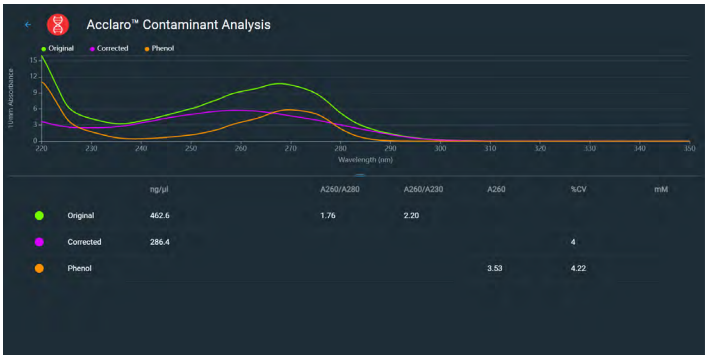

Figure 1: Acclaro contaminant analysis technology screen outlining the original concentration, corrected dsDNA concentration, and the absorbance contribution of phenol contamination. The original spectrum is shown in green, the corrected spectrum is shown in pink, and the phenol spectrum is shown in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

Introduction

Understanding nucleic acid sample quality and quantity is integral for many life science applications and reduces the occurrence of costly delays needed for troubleshooting downstream experiment failures. The NanoDrop Eight 8-channel Microvolume UV-Vis Spectrophotometer provides scientists with the ability to measure the concentration of biomolecules for high-throughput assays using a 1-2 µL sample size. With a measurement time of less than 20 seconds, scientists can easily insert the NanoDrop Eight Spectrophotometer into their high-throughput workflows.

The Acclaro technology built into the NanoDrop Eight instrument PC Software allows for real-time detection of contaminants such as protein and phenol in nucleic acid samples. For example, in qPCR or sequencing workflows, determining sample quality is crucial for a successful experiment. The benefit of detecting contaminants early and in real time saves the time and cost of repeating failed reactions.

Historically the A260/A280 and A260/A230 purity ratios have been utilized to assess sample quality. However, certain contaminants at low concentrations, such as phenol, have a negligible effect on purity ratios, and the contaminant identity is not easily determined by the change in purity ratios. The Acclaro contaminant analysis technology eliminates the need for purity ratio assumptions and reports the contaminant present, contaminant absorbance, and a corrected sample concentration (Figure 1).

Method

Calf thymus DNA (Invitrogen™, 15633019) was spiked with phenol (Fisher BioReagents, BP1750B-65) or bovine serum albumin (BSA) (Sigma Aldrich™, A7284) according to Table 1 and diluted in tris-EDTA buffer (TE, pH 7.6). Each sample was measured using the dsDNA application on the NanoDrop Eight instrument in five replicates with 1.5 µL sample aliquots per pedestal. With TE as the blank solution, the Acclaro Software automatically calculated the absorbance of the contaminant and the corrected dsDNA concentration.

The average corrected concentration and percent error (% error) were calculated for the NanoDrop Eight instrument with the Thermo Scientific™ NanoDrop One instrument as the reference as shown in Table 2. Channel to channel variability data for the NanoDrop Eight instrument are shown in Table 3.

Protein (BSA)		Phenol	
Spike concentration	Target dsDNA concentration	Spike concentration	Target dsDNA concentration
0 mg/ml	500 ng/µl	0 ppm	250 ng/µl
15 mg/ml	500 ng/µl	600 ppm	250 ng/µl
10 mg/ml	500 ng/µl	300 ppm	250 ng/µl
8 mg/ml	500 ng/µl	150 ppm	250 ng/µl
5 mg/ml	500 ng/µl	75 ppm	250 ng/µl
3 mg/ml	500 ng/µl	37.5 ppm	250 ng/µl

Table 1: BSA or phenol was mixed with calf thymus dsDNA and diluted to yield the concentrations listed above.

Results

As shown in Table 2, the Acclaro Software for both the NanoDrop Eight and NanoDrop One instruments calculated the corrected dsDNA concentrations with phenol and protein contamination. The phenol concentration is stated in parts per million (ppm) where a 0.1% solution of phenol is 1000 ppm. With the NanoDrop One instrument serving as the reference spectrophotometer, the % error between the NanoDrop One and NanoDrop Eight instruments was calculated for each protein and phenol spike. The data suggests that the Acclaro technology exhibits high reproducibility between instruments with a % error below 10% and, in most cases, below 5%.

Protein				Phenol			
Sample spike	NanoDrop One Corrected (ng/µl)	NanoDrop Eight Corrected (ng/µl)	% Error	Sample spike	NanoDrop One Corrected (ng/µl)	NanoDrop Eight Corrected (ng/µl)	% Error
15 mg/ml	491.13	479.72	2.32	600 ppm	237.62	258.78	8.91
10 mg/ml	441.20	445.98	1.08	300 ppm	276.80	293.52	6.04
8 mg/ml	446.03	452.57	1.47	150 ppm	278.53	287.09	3.07
5 mg/ml	501.63	506.09	0.89	75 ppm	242.07	248.35	2.59
3 mg/ml	509.50	515.68	1.21	37.5 ppm	266.10	270.94	1.82
Control	523.35	529.53	1.18	Control	249.81	250.53	0.29

Table 2: Comparison of the corrected concentrations calculated by the Acclaro Software from the NanoDrop One and NanoDrop Eight Spectrophotometers. The NanoDrop One instrument served as the reference for % error.

The channel-to-channel variability for the NanoDrop Eight Spectrophotometer was also evaluated using the 3 mg/ml spike sample for protein and the 37.5 ppm spike sample for phenol as examples (Table 3). The variability between channels was low with standard deviations for both protein and phenol below 1.5 ng/µl indicating excellent reproducibility of the Acclaro Software in determining a corrected dsDNA concentration.

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Protein*		Phenol**	
A	517.32	A	271.52
B	516.76	B	272.81
C	515.64	C	270.38
D	513.42	D	271.1
E	515.25	E	270.91
F	517.31	F	269.81
G	514.39	G	270.39
H	515.31	H	270.58
Average	515.68	Average	270.94
Standard Deviation	1.39	Standard Deviation	0.92
%CV	0.27	%CV	0.34

Table 3: Channel to channel (pedestals A-H) variability of the NanoDrop Eight instrument averaged from five replicate measurements per channel.
*** 3 mg/ml protein sample run**
**** 37.5 ppm phenol sample run**

Conclusion

The NanoDrop Eight Spectrophotometer makes sample quality and quantity determination easy and can be seamlessly integrated into workflows such as qPCR and sequencing sample preparation. The inclusion of the Acclaro technology in the NanoDrop Eight instrument software allows users to obtain an accurate sample assessment, eliminating the guesswork of analyzing purity ratios.

The data presented in this note outline the high accuracy and reproducibility of the Acclaro Software in determining phenol or protein contamination across a wide range of concentration spikes for dsDNA samples. The NanoDrop Eight 8-channel UV-Vis Spectrophotometer has been optimized for high-throughput assays. With the inclusion of the Acclaro technology, users can also conserve precious sample volumes and save time and costs by reducing the number of failed reactions due to contamination.