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TECHNICAL NOTE

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Acclaro Protein Contaminant ID

Detection of Protein in Nucleic Acid Samples Using the NanoDrop One Spectrophotometer

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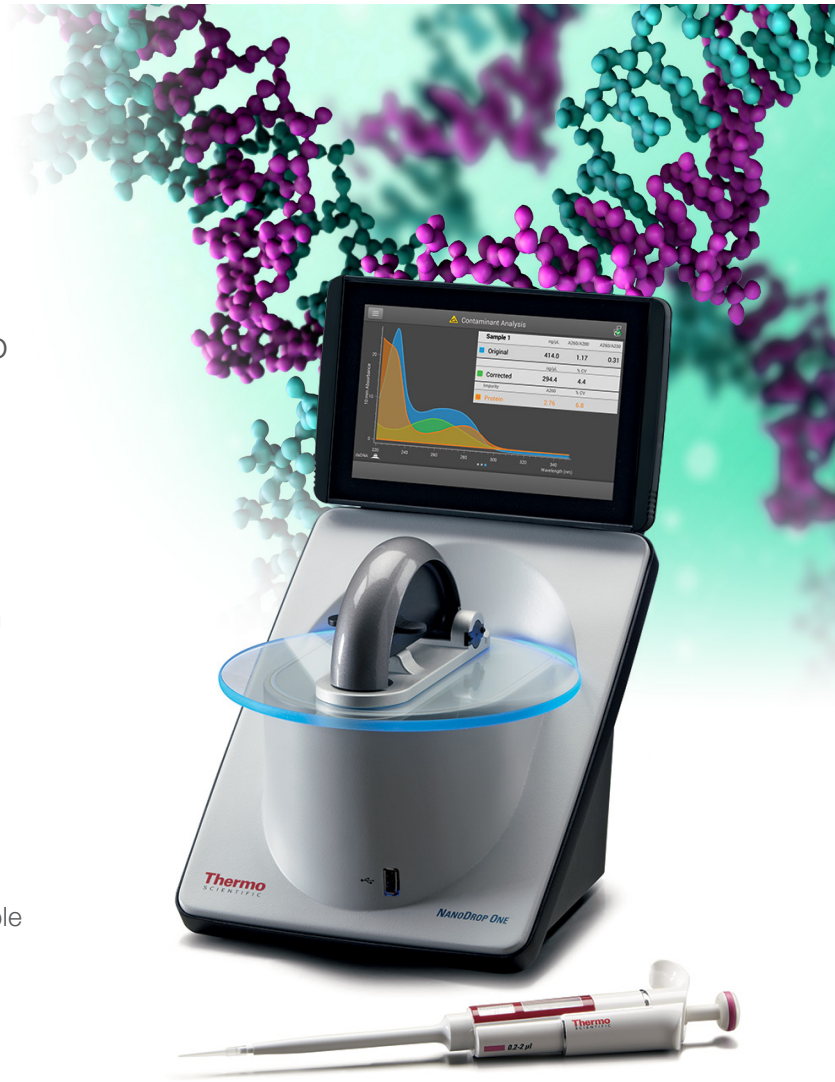

Key Words
NanoDrop One, Acclaro Contaminant ID, Chemometrics, Contaminant Identification, DNA, dsDNA, Nucleic Acid, Protein Contamination, Purity Ratio Quantification, Quantitation, Spectral Analysis, Spectrophotometer, UV-Vis

Abstract
The Thermo Scientific™ NanoDrop™ One microvolume UV-Vis spectrophotometer enables research scientists to accurately quantify nucleic acid or protein samples in the presence of common contaminants. Built with the novel Thermo Scientific™ Acclaro™ Sample Intelligence technology, the NanoDrop One instrument provides more information about sample quality by identifying common contaminants and delivering true sample concentrations. This information enables scientists to make more informed decisions as to how to proceed with downstream experiments and also provides valuable information for troubleshooting problematic extractions. Here we describe how the Acclaro Contaminant Identification (ID) feature detects protein contamination in nucleic acid samples.

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Introduction

The quantification of nucleic acids has traditionally been performed by absorbance measurements at 260 nm (Figure 1). This technique is the method of choice in the molecular biology laboratory, primarily because of the simplicity and ease by which scientists can obtain concentration and purity information about their nucleic acid samples. A consideration when using UV absorbance to evaluate samples is that many contaminants from the nucleic acid extraction process also absorb in various regions of the UV spectrum (Figure 2). Contaminant absorption within the same UV range as nucleic acids can directly affect the quantification result in two ways: it can artificially inflate the A260 value, which results in an inaccurate concentration, and it can affect the purity ratios. For a long time, purity ratios were the main way for researchers to assess the presence of UV-absorbing contaminants. However, relying on purity ratios alone does not provide a complete assessment of the potential contaminants in nucleic acid samples. Purity ratios used in combination with full-spectral data greatly enhance the ability of researchers to determine nucleic acid sample purity and ensure that an accurate concentration can be obtained using an A260 measurement.

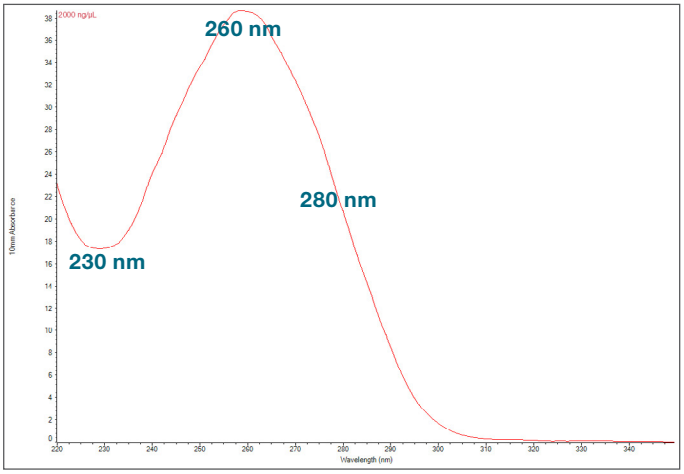

Figure 1: The UV absorbance spectrum of a pure nucleic acid sample has a peak at 260 nm and a trough at 230 nm.

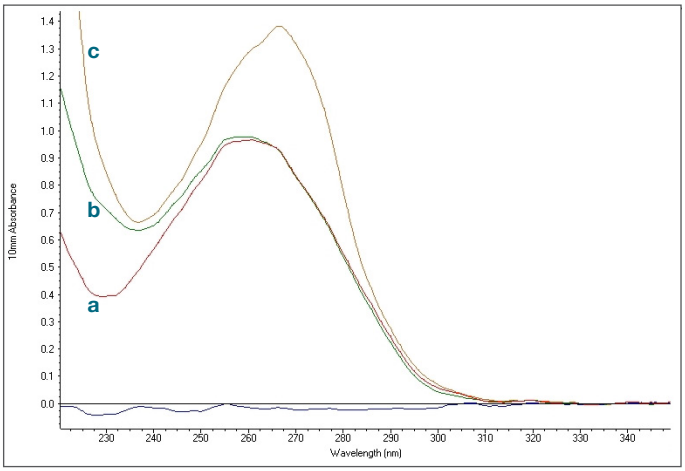

Figure 2: Contaminants can affect the UV absorbance spectrum of a nucleic acid preparation. UV spectrum of a) a pure nucleic acid sample, b) a nucleic acid sample contaminated with guanidine, and c) a nucleic acid sample contaminated with phenol.

In general, researchers will verify that their purity ratios fall into an acceptable range (Table 1). When purity ratios do not fall within the accepted range, the researcher will visually analyze the sample spectrum or seek technical assistance. Until now, analysis of a sample's spectrum has been purely qualitative, and the ability to identify specific contaminants from the spectrum has relied mostly on the experience of the researcher.

Samples	260/280	260/230
DNA	1.8–2.0	1.8–2.2
RNA	2.0–2.2	1.8–2.2

Table 1: Generally accepted purity ratio ranges for "pure" nucleic acid samples in TE buffer.

The Acclaro sample intelligence technology built into the NanoDrop One spectrophotometer provides a quantitative method for contaminant identification by using a chemometric approach to analyze the chemical components present in a sample. Acclaro software uses algorithms that rely on a reference library of spectra. These algorithms are then applied to the sample spectrum, and the software can make predictions about the presence and identity of contaminants by using chemometric mathematical principles. The Acclaro contaminant ID feature can detect protein, phenol, and guanidine salts in dsDNA and RNA samples. The NanoDrop One spectrophotometer alerts users to the presence of a contaminant in real time by displaying the yellow contaminant ID icon (shown in Figure 3a) next to the sample number. Tapping on the contaminant icon reveals the full Acclaro contaminant analysis details (Figure 3b). This screen displays the deconvolved spectra, identified contaminants, corrected DNA concentration, and a %CV, which represents the confidence of Acclaro's algorithms prediction. In this technical note, we present data that illustrates how the Acclaro sample intelligence technology detects protein contamination in nucleic acid samples.

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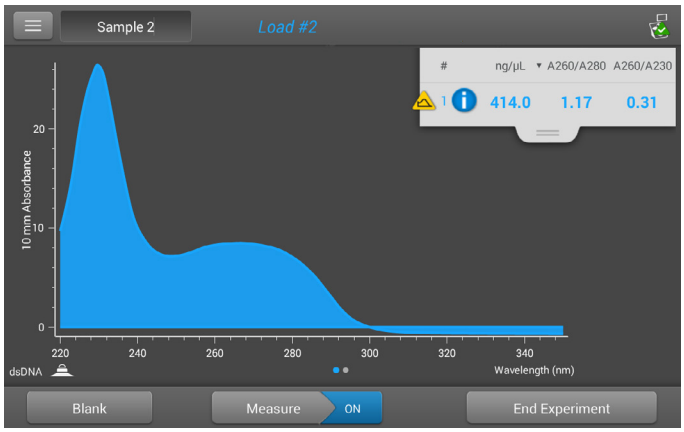

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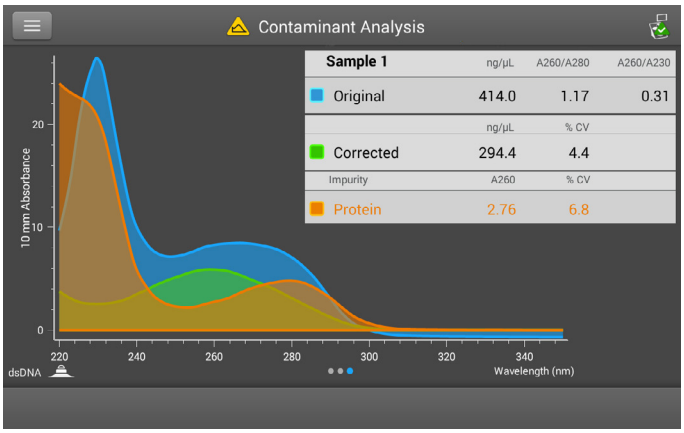

Figure 3: Acclaro Contaminant ID feature alerts the user to possible contaminants present in the sample just measured. 3a) *Measurement screen:* the Acclaro Contaminant ID icon indicates that Acclaro algorithms have detected a contaminant in this dsDNA sample. 3b) *Contaminant Analysis screen*: shows the absorbance spectra of the Original (DNA plus contaminant), Corrected (DNA minus contaminant) and Impurity (identified contaminant) and includes data on concentration, 260/280 and 260/230 ratios. To ensure reproducible results, the Corrected concentration value should be used in planning downstream experiments.

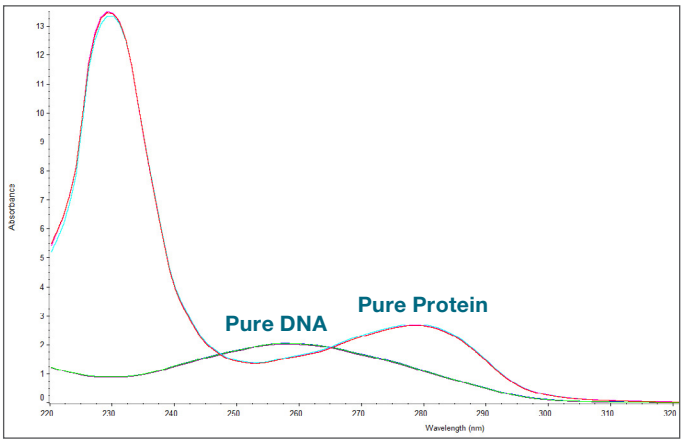

Figure 4: Pure DNA and pure protein spectra overlaid on the same graph. The DNA spectrum (green-blue) has the characteristic peak at 260 nm and trough at 230 nm, whereas, the protein spectrum (red-blue) has the characteristic peak at 280 nm and an increase in absorbance below 250 nm.

Protein Contaminants in Nucleic Acid Samples

Protein as a contaminant in nucleic acid preparations 1) contributes to the absorbance at 260 and inflates the concentration of the nucleic acid and 2) is usually detected by a decrease in the 260/280 purity ratio. The decrease in this ratio occurs because the amino acid residues tryptophan, tyrosine, phenylalanine, as well as the Cystine disulfide bonds absorb light at 280 nm (Figure 4). Most nucleic acid extraction kits ensure an adequate removal of protein; however, many researchers new to phenol/chloroform extractions will often experience protein contamination. During this type of extraction, researchers have to separate the aqueous phase from the organic phase. Because proteins precipitate at the interface between these two phases, it is easy to accidentally bring proteins from the interface into the aqueous phase when performing the nucleic acid extraction, thus introducing protein contamination into their nucleic acid preparation.

The 260/280 ratio was originally used as a very sensitive way to detect DNA contamination in protein preparations (Warburg, 1942).[1] The molecular biology community later adopted the 260/280 ratio as a way to detect protein contamination in nucleic acid preparations. However, the 260/280 ratio, as a means for detecting protein contamination in nucleic acid samples, has its limitations. The extinction coefficients of proteins are very small relative to those of nucleic acids and, therefore, it takes a large amount of protein to affect the 260/280 purity ratio (Glasel, 1995, Huberman, 1995, and Manchester, 1995).[2-4] Nonetheless, scientists still encounter nucleic acid preparations with low 260/280 purity ratios. Protein contamination will not only mislead researchers by inflating the nucleic acid concentration result, but it can also directly affect downstream reverse transcription and qPCR reactions by inhibiting or interfering with enzymatic reactions. In this technical note, we show how the degree of protein contamination affects the purity ratios and quantification results. We also show that the Acclaro Contaminant ID feature of the NanoDrop One spectrophotometer can accurately identify protein as the contaminant and deliver accurate nucleic acid concentration results.

Materials and Methods

Stocks of DNA and protein were prepared as follows. The double-stranded DNA (dsDNA) stock was prepared by diluting a salmon sperm DNA solution (Invitrogen™, #15632-011) in Tris-EDTA (TE) buffer (Fisher BioReagents™, pH 7.6, BP-2474-500). The protein stock was prepared by diluting a solution of bovine serum albumin (BSA, Sigma Aldrich®, #A7284) in TE buffer. The concentrations of both dsDNA and BSA stocks were determined on the NanoDrop One spectrophotometer against a TE blank. Mass calculations were made using the factor 50 ng-cm/µL for dsDNA and the extinction coefficient E1% 6.7 for BSA. Nine mixtures of DNA and protein were then prepared by adding various amounts of the DNA and protein stocks to generate the mixtures shown in Table 2.

Five replicates of each solution 1–9 were measured on the NanoDrop One spectrophotometer against a TE blank. A fresh 1.5 µL aliquot of the appropriate mixture was used for each replicate. The software calculated concentrations (corrected and original/uncorrected) and Acclaro Contaminant identity data, were then used to generate the data sets presented in Table 3:

- Average concentrations and standard deviation (SD) based on uncorrected concentrations
- Average concentration and SD based on Acclaro corrected concentrations
- Average purity ratios for each mixture

The original (uncorrected) concentrations versus the Acclaro-corrected concentrations were compared, and results are discussed below. Since mixtures 1–4 did not contain high enough protein levels to trigger an Acclaro result, the corrected DNA concentration for mixtures 1 through 4 were determined by performing the Acclaro spectral analysis using the Thermo Scientific™ TQ Analyst™ software package.

Mixture	%DNA (by mass)	% Protein (by mass)
1	100.0	0.0
2	57.1	42.9
3	40.0	60.0
4	28.6	71.4
5	16.7	83.3
6	10.0	90.0
7	7.1	92.9
8	4.8	95.2
9	1.6	98.4

Table 2: DNA and protein stocks were mixed to yield the proportions shown above.

Mixture	1	2	3	4	5	6	7	8	9
% Protein (by weight) in Mixture	0.0	42.9	60.0	71.4	83.3	90.0	92.9	95.2	98.4
Original (Uncorrected) DNA [conc] ng/µL	531.8	534.7	542.1	571.5	591.3	644.4	672.9	740.4	1097.6
Corrected DNA [conc] ng/µL	526.8	523.9	525.9	540.4	541.1	554.6	549.2	547.3	560.1
260/280 Purity Ratio	1.94	1.89	1.84	1.74	1.60	1.45	1.34	1.21	0.89
260/230 Purity Ratio	2.45	1.67	1.34	0.90	0.61	0.41	0.32	0.26	0.19
Acclaro Flag	No	No	No	No	⚠	⚠	⚠	⚠	⚠

Table 3: The uncorrected (original) DNA concentration of each mixture was determined with the NanoDrop One spectrophotometer using the dsDNA application. The corrected DNA concentration for mixtures 5–9 was obtained directly from the NanoDrop One Acclaro contaminant analysis results. The Acclaro contaminant ID icon(⚠) denotes mixtures that have levels of protein contamination high enough to trigger an Acclaro result.

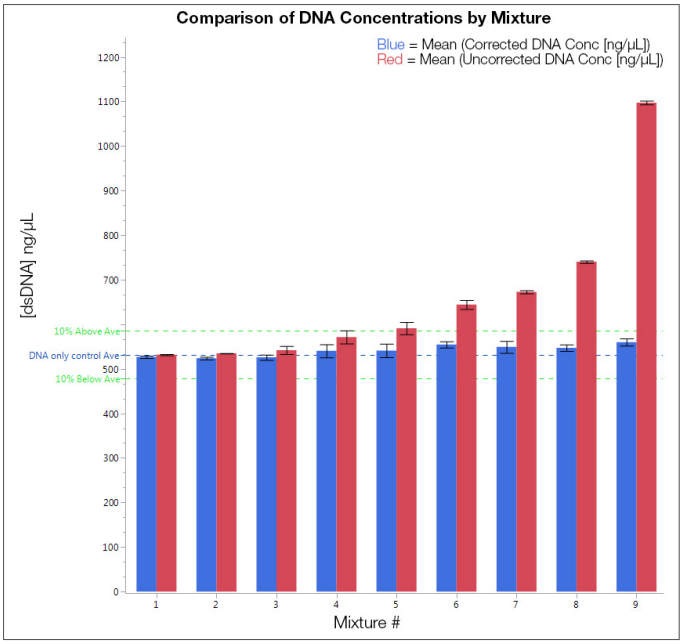

Figure 5: The Acclaro-corrected concentration is within 10% of the actual DNA concentration for all mixtures. Red bars represent the uncorrected (original) DNA concentrations. The blue bars represent the corrected DNA concentrations reported by Acclaro software (or calculated for mixtures 1–4 as described in Materials and Methods). The dotted blue reference line is the concentration for the DNA-only control (mixture #1 – the actual dsDNA concentration). The dotted green reference lines represent either 10% above or below the DNA-only control average, 531.8 ng/µL. Each data point represents the average of five measurements. Error bars represent 1 standard deviation from the mean.

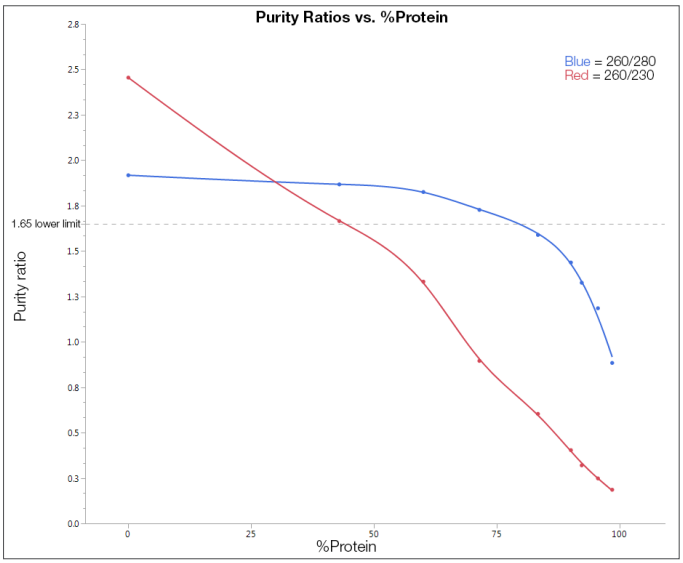

Figure 6: In this graph the average purity ratios are plotted for each dsDNA/Protein mixture (mixtures 1–9). The red line denotes the 260/230 purity ratio, and the blue line denotes the 260/280 ratio.

Results and Discussion

Table 3 presents the Acclaro Contaminant ID data obtained for the nine DNA/protein mixtures described in Table 2. As the level of protein contamination increases, the discrepancy between the corrected and the original (uncorrected) results becomes larger. This emphasizes how protein contamination can inflate an A260 concentration result. The Acclaro-corrected results show how the software algorithm can quantitatively correct for these levels of protein contamination and provide a more accurate DNA concentration than the A260 value alone. The data in Table 3 also demonstrate that a large amount of protein contamination is required before a significant change in the 260/280 purity ratios is observed. Samples with as much as ~72% protein by weight still have an acceptable 260/280 purity ratio. As the protein contaminant levels increase from ~72% to 98%, the 260/280 ratio drops steadily from 1.74 to 0.89.

The bar graph in Figure 5 shows a comparison between the uncorrected and corrected DNA concentration data in the presence of different levels of protein contaminant. The presence of protein can inflate the original reported concentration. The data also shows that when the contaminant concentration is above ~72% protein by weight, Acclaro flags the sample and displays a corrected concentration (Table 3). The corrected DNA concentrations are within 10% of the result for the DNA-only control. Mixture 9, which has the highest amount of protein contamination, shows the largest difference between the corrected and uncorrected concentration results. However, even with this extremely contaminated mixture where the protein represents >98% of the analyte mass in the sample, the Acclaro-corrected result brings the concentration result for mixture 9 to within 10% of the actual DNA concentration. These results were highly reproducible with standard deviations averaging under 1 ng/µL.

The graph in Figure 6 shows how purity ratios change with increasing levels of protein. As expected, as the percentage of protein contamination increases, the 260/280 purity ratio decreases. However, as shown in Figure 6, the amount of protein has to be larger than 75% of the sample to observe a significant decrease in the 260/280 purity ratio (below 1.65), which is the lower limit generally accepted for use in downstream experiments. On the other hand, the 260/230 purity ratio steadily decreases as the percentage of protein in the mixture increases. Figure 6 indicates that the

260/230 ratio may be a more sensitive indicator of protein contamination. The challenge of using this ratio to detect protein contamination is that many other common contaminants, such as common salt buffers, guanidine salts, and polysaccharides, can affect this ratio as well. Therefore, the 260/230 ratio alone cannot confirm protein contamination. The Acclaro Contaminant ID feature of the NanoDrop One spectrophotometer delivers a definitive advantage to the researcher by providing a corrected DNA concentration and by identifying contaminants present in a nucleic acid sample.

Conclusion

Experiments that use nucleic acids require that the concentration and purity of the sample is known. The UV-Vis method used for the quantification of nucleic acid preparations relies on the absorbance of nucleic acid molecules at 260 nm to determine the concentration of nucleic acids in solution. Contaminants such as proteins that are co-purified with nucleic acids can also absorb light in the UV region of the spectrum, which leads to overestimating the calculated nucleic acid concentration.

Traditionally, researchers have relied on purity ratios as an indication of the presence of contaminants in nucleic acid samples. “Out of range” purity ratios can inform users of the presence of contaminants, but they do not provide information on the identity and amount of the contaminant present. The Acclaro sample intelligence technology in the NanoDrop One spectrophotometer provides a chemometric approach for contaminant identification using UV spectrum analysis. This feature empowers researchers by helping them 1) identify the type of contaminant present in their sample 2) determine the level of contamination and 3) obtain a corrected nucleic acid concentration. By using the Acclaro sample intelligence technology, researchers are now able to make informed decisions on how to troubleshoot sample preparations to reduce contamination and how to proceed using a sample in downstream experiments.

In this application note, we show how the Acclaro Contaminant ID feature:

- Accurately identifies protein as the contaminant present in nucleic acid samples
- Accurately calculates the amount of protein contamination present in a nucleic acid sample
- Provides corrected concentration results that are more accurate than using absorbance at 260 nm alone. Even at very high levels of protein contamination, the corrected values are within 10% of the actual nucleic acid concentration.

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