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TECHNICAL NOTE

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NanoDrop One sample contaminant identification

Frequently Asked Questions

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The Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer is designed to help research scientists achieve success in downstream applications by accurately quantifying DNA, RNA and protein samples using only 1-2 µL. Built with 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. Here are answers to common questions about the Acclaro Contaminant Identification (ID) feature.

1. How does the Acclaro Contaminant ID feature work on the NanoDrop One Spectrophotometer?

The Acclaro Contaminant ID feature uses a chemometric approach to analyze the chemical components present in a sample. This type of analysis has been used previously in Fourier transform infrared spectroscopy (FTIR) and near-infrared systems (e.g., Thermo Scientific™ Nicolet Spectrometers). The Acclaro algorithms rely on a reference library of spectra. These algorithms are then applied to the sample spectrum, and the software can make predictions about a sample's contaminants by using chemometric mathematical principles.

2. In which NanoDrop One application is the Acclaro Contaminant ID feature implemented?

Contaminant ID is implemented in the dsDNA, RNA and Protein A280 Applications.

3. What contaminants can the Acclaro Contaminant ID technology detect?

The Acclaro reference libraries currently support detection of protein, phenol, and guanidine HCl in dsDNA samples. They also support detection of protein, phenol, and guanidine isothiocyanate in RNA samples. Finally, they allows detection of DNA in protein samples. In the future, additional contaminants will be added to the libraries.

4. Are the Acclaro Contaminant ID algorithms applied to all sample concentrations?

No, in the dsDNA and RNA applications, the Acclaro Contaminant ID algorithms are only applied to samples whose absorbance at 260 nm falls between 0.5A and 62.5A. In the protein A280 application, the Acclaro Contaminant ID algorithms are applied to all samples regardless of concentration. The table to the right summarizes common contaminants detected by Acclaro sample intelligence technology.

	NanoDrop One Application		
Acclaro technology applied to:	**dsDNA**	**RNA**	**Protein**
Detection Wavelength	260 nm	260 nm	280 nm
Sample Concentration	0.5A-62.5A 25-3,125 ng/µL	0.5A-62.5A 20-2,500 ng/µL	All concentrations
Detected Contaminants	Protein Phenol Quanidine HCl	Protein Phenol Guanidine Isothiocyanate	DNA

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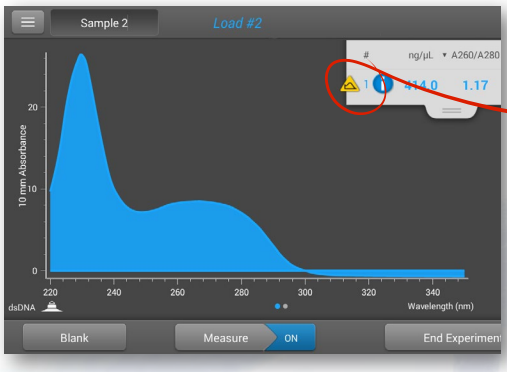
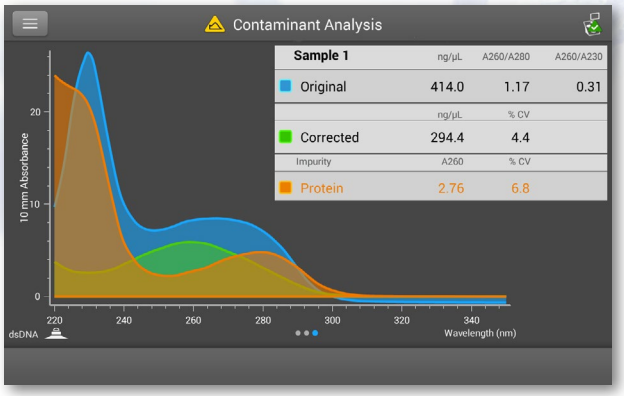
5. How do you read the Acclaro Contaminant ID results?



Acclaro Contaminant ID icon:
The Acclaro algorithms have detected a contaminant in this dsDNA sample.

This Results screen shows that the Acclaro algorithms have detected contaminants in this DNA sample, as represented by the yellow triangle. To find out what contaminants the Acclaro software has identified and to obtain the corrected DNA concentration, simply tap the yellow icon to open the Contaminant Analysis screen shown below.



Original:
Concentration result without any Acclaro Contaminant correction applied. *Blue Spectrum*

Corrected:
Concentration result with Acclaro Contaminant correction applied. *Green Spectrum*

Impurity:
Contaminant that has been detected and how much 260 nm absorbance the contaminant contributed to 260 nm peak. *Orange Spectrum*

The Acclaro Contaminant Analysis screen presents important information about the sample. The original concentration and purity ratio results are shown on the top row. This is the calculated concentration result (Original) before contaminant corrections have been applied. The second row labeled “Corrected” shows two values. The first value is the corrected concentration of the analyte in the sample in ng/µL (the analyte in this example is dsDNA as indicated in the lower left corner of the screen). The Corrected result is the analyte concentration after contaminant correction has been applied. This value is the software-predicted concentration for the pure nucleic acid component in the sample. The second value is the %CV, which represents the confidence in the prediction. The lower the %CV number, the more confident we are in the corrected concentration predicted by the software. The third row identifies the impurity that is present (protein, phenol, guanidine HCl, or guanidine isothiocyanate) and reports how much absorbance at 260 nm is contributed by the contaminant. In this case, the %CV represents the confidence in the contaminant identity prediction.

6. How do we use Acclaro Contaminant ID results in workflows?

The Acclaro Contaminant ID feature gives customers two important pieces of information. First, the Contaminant ID feature identifies specific contaminants that are likely to be present in the sample. The identification of the potential contaminants in the sample can help scientists troubleshoot difficult extractions or purifications and make decisions regarding sample use in downstream experiments. Second, the Contaminant ID gives customers a corrected concentration. When setting up downstream reactions where DNA concentration is a critical parameter (e.g., PCR), the corrected concentration will help scientists ensure the success of downstream experiments.

7. What accuracy and sensitivity can I expect from the Acclaro Contaminant ID feature, and what factors affect the accuracy of the result?

Large sets of known mixtures of analytes “spiked” with contaminants were measured on the NanoDrop One instrument during the development of this feature. In general, the corrected nucleic acid result (the software-predicted concentration) was within 10% of the actual concentration. The sensitivity of Acclaro Contaminant ID depends on the contaminant’s molar absorptivity relative to the analyte of interest (nucleic acid or protein). Phenol has a very high molar absorptivity, therefore, it takes very little phenol to affect the spectrum and generate a corrected concentration for the analyte. Protein has a low molar absorptivity relative to dsDNA, therefore, it takes a large amount of protein to affect the spectrum.

Nous contacter

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