Technical Note

#4 Nucleic Acids Performance Data

Introduction

Nucleic Acid quantification is an important step in many different life science protocols including those implemented for NGS, qPCR and many other areas. The NanoPhotometer® offers a preprogrammed Nucleic Acid method with options to measure all types of nucleic acids like dsDNA, ssDNA, RNA, miRNA, Oligo, options to enter sequences for miRNAs and Oligos as well as a custom defined extinction coefficient for other types of nucleic acids. It is also possible to measure labeled nucleic acids via the absorbance maxima of the dye. Dye concentration and the frequency of incorporation are calculated and displayed automatically.

In this technical note we describe the performance of the NanoPhotometer® NP80 in terms of linearity and accuracy, applying small volume nucleic acid quantification at 260 nm. Nucleic acid samples display a characteristic absorption spectrum at 260 nm. The Lambert-Beer law can be applied to determine the nucleic acid concentration in a sample. Along with the nucleic acid concentration, the NanoPhotometer® NP80 also assesses the purity of the nucleic acid sample by calculating the 260/230 and 260/280 ratios. The NanoPhotometer® features Blank Control™ that will automatically warn the user if high background is present in a blank from buffers or contaminants (see also Technical Note #3 Blank Control™).

Material & Methods

DNA from fish sperm 74782 from Sigma (Lot. 1343783 was used to create aqueous stock solution with 30,000 ng/µL. Different sample concentrations were achieved by dilution with distilled water. Dilution ratio was controlled by weight via microbalance (Satorius BP 221S). Expected absorbance values were determined in 10 mm quartz glass cuvettes (Hellma Analytics 100-QS) with a calibrated UV/Vis spectrometer UVIKON XL (serial number 110178). All sample measurements were done using NanoPhotometer® NP80 (serial number M80798).

DNA concentrations were measured five times using a sample volume of 1 µl. Samples were vortexed before each measurement to ensure homogeneity. After each measurement, the pedestal and the lid were cleaned with a slightly wet lint free tissue and a new aliquot of the sample was pipetted.

Accuracy Results

In Table 1 the expected DNA concentration and the mean value of the five measurements of each DNA concentration is shown. Additionally the standard deviation of the mean absorbance at the 0.67 mm / 0.07 mm path of each sample is listed.

Table 1: Mean is out of five different measurements; SD of mean absorbance (0.67 mm path / 0.07 mm path).

Expected ng/µl	Measured ng/µl	SD	Path length
1.89	1.62	0.0005	0.67 mm
3.44	4.86	0.0031	0.67 mm
7.99	9.62	0.0008	0.67 mm
15.51	16.74	0.0002	0.67 mm
29.55	31.26	0.0003	0.67 mm
60.11	61.05	0.0005	0.67 mm
117.66	117.91	0.0008	0.67 mm
234.43	232.77	0.0022	0.67 mm
468.71	467.93	0.0050	0.67 mm
932.01	940.16	0.0075	0.67 mm
1,863.91	1,936.84	0.0033	0.07 mm
3,711.00	3,809.34	0.0072	0.07 mm
7,369.30	7,521.32	0.0233	0.07 mm
14,711.00	15,469.20	0.0285	0.07 mm

Ratio

The NanoPhotometer® NP80 calculates the 260/230 and 260/280 ratios which give information about contaminants of the sample. The 260/230 ratio should be > 2.0 lower ratios indicate a contamination with e.g. guanidinum thiocyanate or other buffer salts (TRIS, EDTA) used during the nucleic acid isolation/purification. The 260/280 ratio indicates the presence of proteins in the nucleic acid sample. Pure DNA and RNA preparations have expected ratios of ≥ 1.8 and ≥ 2.0, respectively.

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Linearity Results

The resulting linearity curve in the range of 1.89 – 14,711 ng/µl shows a close correlation between expected and measured concentrations with coefficient of determination (R^2) of 0.9998 (Figure 1).

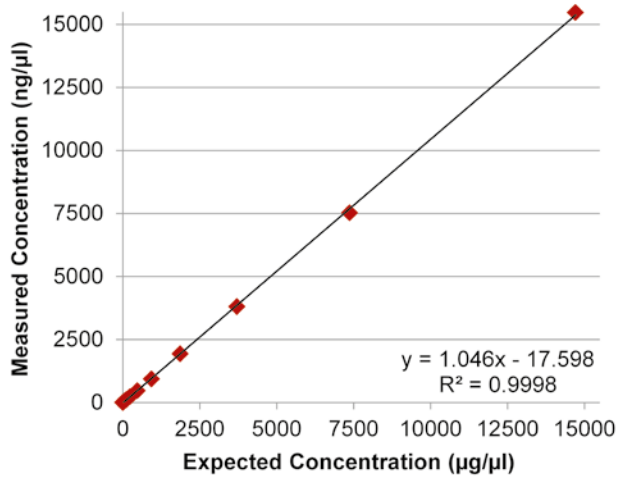

Figure 1: Samples (mean value) in the range of 1.89 – 14,711 ng/µl were measured with automatic path length selection.

Figure 2 shows the linearity for low concentrated DNA samples measured with path length 0.67 (dilution factor 15).

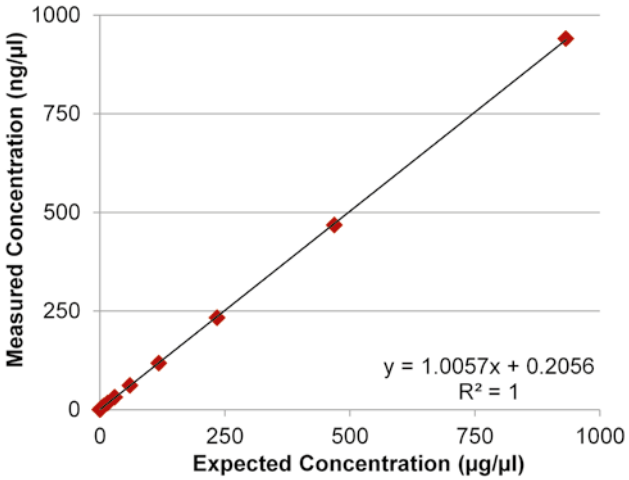

Figure 2: Samples (mean value) in the range of 1.89 – 932 ng/µl were measured with path length 0.67 mm.

Figure 3 shows the linearity of high concentrated DNA samples in the range of 1,863.9 – 14,711 ng/µl (dilution factor 140).

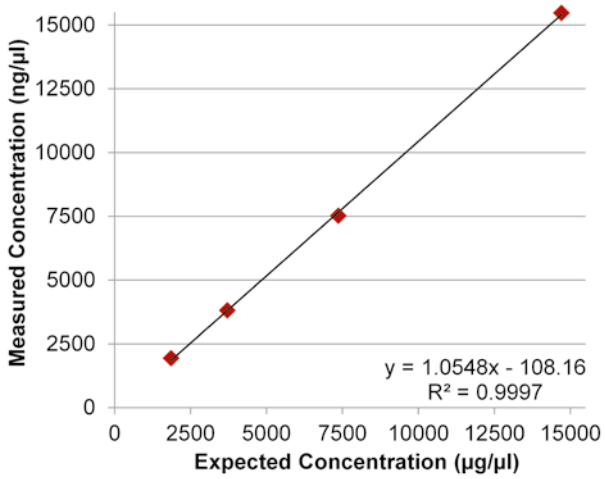

Figure 3: Samples (mean value) in the range of 1,863.9 – 14,711 ng/µl were measured with path length 0.07 mm.

Discussion

This technical note shows excellent linearity of the NanoPhotometer® with a coefficient of determination (R^2) of 0.9998 over the whole dynamic range for DNA samples. Furthermore, the data prove that two precisely defined path lengths are sufficient for this excellent precision and linearity. The sealed mechanical setup of the NanoPhotometer® with two fixed anchor points guarantees accurate performance every time a sample is measured.

Whilst in Figure 1 the whole dynamic range is being analyzed, Figure 2 and 3 focus on low/high concentrated samples. The results show very clearly that across the detection limits of the NanoPhotometer® NP80 precision and linearity of the instrument is highly reliable.

Summary

The data presented in this technical note show the high accuracy and linearity of the NanoPhotometer® across the entire dynamic range of the instrument. With this novel approach of only two precise path lengths with fixed anchor points utilizing the proprietary True Path Technology™ in a sealed optical environment, mechanical changes in the system are eliminated. For further technical information refer also to Technical Note #1 True Path Technology™. The NanoPhotometer® is the only instrument with True Path Technology™ providing accurate results without the need for recalibration throughout the entire lifetime of the instrument.

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