Datasheet

NanoDrop Ultra fluorescence performance data

Introduction

The Thermo Scientific™ NanoDrop™ Ultra FL/Ultra^{C} FL Spectrophotometer and Fluorometer measures the fluorescence emission of a dye bound to dsDNA or RNA to calculate concentration. The Thermo Scientific™ NanoDrop™ Ultra dsDNA Broad Range (BR) and RNA BR assay kits require only two standards, which are applied to a curve-fitting algorithm to convert relative fluorescence units (RFU) to concentration. Both assays can accurately quantify sample concentrations from 10 ng/µL to 1000 ng/µL using either 1.0 µL or 2.0 µL sample volumes in the assay, depending on the original sample concentration.

Method

The NanoDrop Ultra dsDNA BR and RNA BR assay kits are supplied with a 100 ng/µL standard and a 0 ng/µL standard. Dilutions of the 100 ng/µL standard were performed using tris-EDTA (TE) pH 7.5 to obtain sample concentrations of 75, 50, 25, and 10 ng/µL. To test the extended range of both assays (100 – 1000 ng/µL), total RNA from human lymphocytes (BioChain, R1254148-1, ~2,600 ng/µL stock) and lambda DNA (Invitrogen™ lambda DNA, 25250010, ~500 ng/µL stock) were diluted in TE pH 7.5. Standards and samples were mixed with freshly prepared working solution as stated per the kit manufacturer's specifications.

Five replicates of the standards were measured to create a standard curve plot, from which sample concentrations were determined. Samples were measured using fresh 2.0 µL aliquots on the microvolume pedestal in replicates of five. The replicates were averaged, and performance was assessed by calculating the coefficient of variation (%CV) and percent error (% error) compared to theoretical concentrations. Specification limits for %CV and % error for both dsDNA and RNA BR assays are 20% and 15% respectively.

Results

dsDNA BR

The five dsDNA replicate sample concentrations are outlined in Table 1. The average concentration of the five replicates for each dilution was within the % error specification, with all samples displaying less than 9% error compared to the theoretical concentration. Percent CV was less than 3%, indicating exceptional measurement to measurement reproducibility. The linearity of the reported concentrations versus the theoretical concentrations is displayed in Figure 1. The R^2 of the regression line, 0.9992, confirms the measured concentration is highly correlated with the theoretical values.

RNA BR

Table 2 displays the RNA replicate concentrations reported by the NanoDrop Ultra FL instrument. The % error of reported concentrations compared to theoretical was under 9% and the %CV was less than 4% across all samples. In Figure 2, a linearity assessment comparing the theoretical RNA concentrations with those reported by the NanoDrop Ultra FL instrument shows a strong correlation, as evidenced by a regression line with an R^2 value of 0.9998. These results demonstrate that the NanoDrop Ultra FL instrument provides accurate and reproducible RNA concentration measurements across replicates.

Theoretical sample concentration	400 ng/µL	200 ng/µL	100 ng/µL	75 ng/µL	50 ng/µL	25 ng/µL	10 ng/µL
Replicate 1	370.81	181.54	103.98	77.02	50.52	22.61	9.21
Replicate 2	376.79	184.62	99.25	74.51	48.88	23.43	9.19
Replicate 3	372.42	189.18	102.49	73.17	47.26	23.30	8.85
Replicate 4	372.63	190.89	104.08	74.97	48.83	22.86	9.35
Replicate 5	371.29	188.41	104.69	71.05	49.00	22.64	9.23
Average	372.79	186.93	102.90	74.14	48.90	22.97	9.17
%CV	0.63	2.02	2.13	2.98	2.36	1.65	2.06
Theoretical %Error	6.80	6.54	2.90	1.14	2.20	8.14	8.35

Table 1. Average, %CV, and % error to the theoretical concentration for dsDNA samples measured with the NanoDrop Ultra dsDNA Broad Range kit.

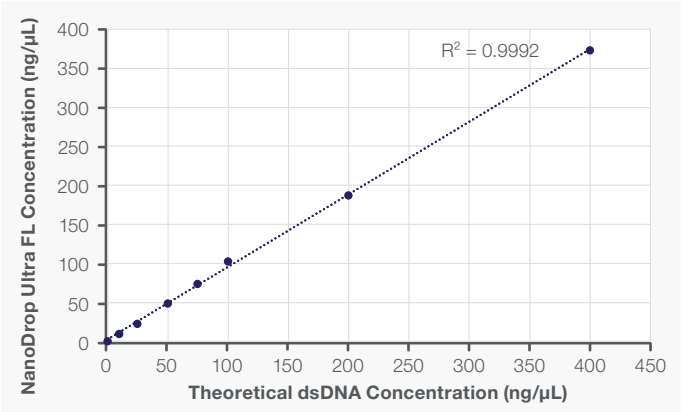

Figure 1. Linearity comparison between the theoretical dsDNA concentration and the reported NanoDrop Ultra FL instrument concentration. The regression line (R^2 = 0.9992) indicates a strong correlation between theoretical and measured concentrations.

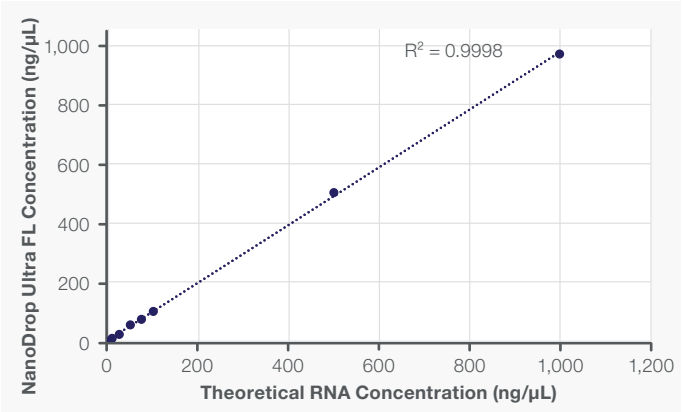

Figure 2. Linearity comparison between the theoretical RNA concentration and the reported NanoDrop Ultra FL instrument concentration. The regression line (R^2 = 0.9998) confirms a strong correlation between theoretical and measured concentrations.

Theoretical sample concentration	1000 ng/µL	500 ng/µL	100 ng/µL	75 ng/µL	50 ng/µL	25 ng/µL	10 ng/µL
Replicate 1	1000.03	494.30	104.10	76.84	54.21	24.70	10.44
Replicate 2	961.73	506.13	103.95	72.36	55.92	24.78	10.02
Replicate 3	979.85	505.81	100.00	78.73	54.60	24.33	10.63
Replicate 4	983.24	496.34	101.10	73.98	52.12	23.85	10.24
Replicate 5	944.45	512.14	97.62	73.98	53.99	24.43	10.09
Average	973.86	502.95	101.36	75.18	54.17	24.42	10.29
%CV	2.19	1.48	2.71	3.40	2.53	1.50	2.45
Theoretical %Error	2.61	0.59	1.36	0.24	8.34	2.32	2.86

Table 2. Average, %CV, and % error to the theoretical concentration for RNA samples measured with the NanoDrop Ultra RNA Broad Range kit.

Conclusions

The NanoDrop Ultra FL/Ultra^{C} FL spectrophotometer and fluorometer measures a broad concentration range of dsDNA and RNA using fluorescence assays without requiring costly consumables or large sample volumes, reducing both time and expenses. Using only 1.0 µL or 2.0 µL of sample in the assay, concentrations within the range of 10 ng/µL to 1000 ng/µL can be quickly quantified without the need for error-prone dilutions. The NanoDrop Ultra dsDNA BR and RNA BR assays have demonstrated high reproducibility between measurements and yielded accurate sample concentrations.

The absorbance applications available on the NanoDrop Ultra spectrophotometers and fluorometers are recommended to determine purity in addition to concentration via fluorescence as contaminants contribute to failed downstream reactions.[1-2] The NanoDrop Ultra FL/Ultra^{C} FL spectrophotometer and fluorometer provides a fast, all-in-one assessment of nucleic acid concentration and purity that minimizes the time needed for troubleshooting at the bench.

References

1. Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors – occurrence, properties and removal. *Journal of Applied Microbiology, 113*(5), 1014–1026. https://doi.org/10.1111/j.1365-2672.2012.05384.x
2. Thermo Fisher Scientific. (2022). *Using NanoDrop One/OneC to determine phenol and protein contaminants in nucleic acids for RT-qPCR quality control.*

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