

Technical Note

COMPARING MICROVOLUME SPECTROPHOTOMETERS

COLIBRI+ VS NANODROP™ ONE, DS-11 AND NABI

Abstract

The Colibri+ LB 916 Microvolume Spectrophotometer features short measurement times, broad concentration and wavelength range, as well as an optimized sample area. Its accuracy and reproducibility are comparable to the top competitors (NanoDrop™ One and DS-11), even surpassing them at some concentrations.

Introduction

Reliable and accurate quantification of DNA, RNA and proteins is needed for many downstream methods, such as qPCR, cloning, NGS, western blotting, and more. Absorbance has been the method of choice for routine quantification of DNA and RNA since decades [1]. It is simple and convenient to use, as no further sample treatment (other than DNA extraction) or reaction with other

substances is required. Available sample volume is often very limited in many Molecular Biology methods, and this makes microvolume spectrophotometers, which allow the measurement of tiny drops of sample (typically 1-2 μL), the instrument of choice for quantification of DNA, RNA and proteins using absorbance.



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There are many microvolume spectrophotometers available on the market. In this technical note, we compare the performance of the Colibri+ LB 916 with three other instruments, the NanoDrop™ One from Thermo Fisher Scientific and the DS-11 from DeNovix, and with the Nabi from MicroDigital.

Colibri+ Microvolume spectrophotometer

The Colibri+ is a high-performance microvolume spectrophotometer for the determination of the concentration of DNA, RNA, and proteins in microvolumes (typically 1 μL) using absorbance.

The system has been designed to help you accelerate your research:

- Ultra-fast measurement (<3 s/sample).
- Intuitive operation.
- High reliability (CV < 1%).
- Broad detection range (2-20,000 ng/ μL ds DNA).
- Broad wavelength range (190-1000 nm).
- Easy pipetting of samples thanks to its Sample Guide Light.



Whether you are performing real-time PCR, sequencing/NGS or cloning experiments, the innovative features of the Colibri+ will help you to advance your application.

Materials

- Colibri+ from Berthold Technologies (Id. Nr. 73179).
- NanoDrop™ One from Thermo Fisher Scientific (Cat # ND-ONE-W).
- DS-11 from Denovix.
- Nabi from MicroDigital.
- Salmon Sperm DNA, sheared (10 mg/mL) from Invitrogen™ (Cat # AM9680).
- Nuclease-free water to prepare the dilution series of DNA.
- Pipettes and pipette tips of various sizes.

Methods

A dilution series of dsDNA was prepared by serially diluting $\frac{1}{2}$ the stock solution (10 mg/mL). This resulted in the following concentrations: 10000, 5000, 2500, 1250, 625, 312.5, 156.25, 78.13, 39.06, 19.53, 9.77, 4.88, and 2.44 ng/ μL .

Each DNA concentration was measured in triplicate with all instruments. In order to reduce the contribution of pipetting error, the volume measured was 2 μL in all cases.

The following parameters were calculated:

- To assess the accuracy of the instruments, the absolute value of the difference between the measured dsDNA concentration and the theoretical concentration was calculated and expressed as percent of the theoretical concentration. This parameter is displayed as Error (%) in the tables.
- To assess the reproducibility of the instruments, the coefficient of variation of the measured dsDNA concentration was calculated (standard deviation expressed as percent of the theoretical concentration). This parameter is displayed as CV (%) in the tables.

Instrument settings

The measurement was performed with default settings for dsDNA in all instruments, with baseline correction active. In the Colibri+, a pathlength of 0.5 mm was used for concentrations of 625 ng/μL and

lower, and 0.05 mm for concentrations of 1250 ng/μL and higher.

Results

Reproducibility results are summarized in Table 1. The reproducibility of the Colibri+ is comparable to that of the NanoDrop™ One and DS-11 in the whole concentration range, with excellent CV% values at concentrations above 20 ng/μL and is better than that of the Nabi. In all instruments, CV% increases notably at the lowest concentrations.

Accuracy results are summarized in Table 2. As shown by the calculated errors, the accuracy of the Colibri+ is better than that of the other instruments tested at concentrations of 312.5 ng/μL and below (with the exception of 4.88 ng/μL). Error is considerably high in all instruments at low concentrations, and it increases as the concentrations approach the lowest concentrations. In fact, some instruments (DS-11 and Nabi) already exhibit errors above 10% at concentrations of 39.06 ng/μL, while in the Colibri+, this happens only at the lowest concentrations (9.77 ng/μL and below). At higher concentrations the error is comparable in all instruments, and typically under 5%.

ng/μL dsDNA	CV% Colibri+	CV% ND One	CV% DS-11	CV% Nabi
10000	0.4	1.7	0.2	8.7
5000	0.3	0.8	0.3	6.9
2500	0.5	0.2	0.4	0.7
1250	0.4	1.5	0.3	1.0
625	0.1	0.3	0.1	0.8
312.50	0.1	0.1	0.1	0.9
156.25	0.6	1.2	0.5	0.3
78.13	1.2	0.3	1.0	0.9
39.06	0.6	0.1	1.7	1.6
19.53	2.1	1.0	2.2	0.6
9.77	5.8	1.9	5.9	3.3
4.88	8.6	5.7	7.1	8.0
2.44	14.2	15.2	13.4	21.6

Table 1: Reproducibility of the measurement of dsDNA concentrations. All measurements in triplicate.

ng/ μ L dsDNA	Colibri+		NanoDrop™ One		DS-11		Nabi	
	Measured	Error (%)	Measured	Error (%)	Measured	Error (%)	Measured	Error (%)
10000	9557.4	4.4	9489.9	5.1	10300.7	3.0	10462.3	4.6
5000	4650.9	7.0	5248.6	5.0	5047.5	0.9	5572.3	11.4
2500	2435.6	2.6	2504.6	0.2	2485.7	0.6	2618.8	4.8
1250	1254.7	2.5	1276.1	2.1	1305.7	4.5	1370.0	9.6
625	657.1	5.1	639.5	2.3	651.3	4.2	655.5	4.9
312.50	316.0	1.2	293.9	6.0	327.3	4.9	332.0	6.3
156.25	163.6	4.9	146.6	6.2	169.5	8.6	169.8	8.7
78.13	81.4	4.4	72.0	7.8	84.5	8.3	84.7	8.4
39.06	42.1	7.9	35.5	9.2	44.4	14.0	44.8	14.6
19.53	21.9	9.3	16.0	18.3	23.0	14.9	24.9	27.7
9.77	11.3	12.6	8.1	17.0	11.6	16.0	14.1	44.7
4.88	6.3	25.8	4.1	16.5	5.9	17.8	8.0	64.5
2.44	3.3	30.9	1.5	40.2	3.9	55.4	4.6	89.8

Table 2: Accuracy in the measurement of dsDNA concentrations. Error was calculated as the absolute value of the difference between the measured concentration and the theoretical concentration, expressed as percent of the theoretical concentration. All measurements in triplicate.

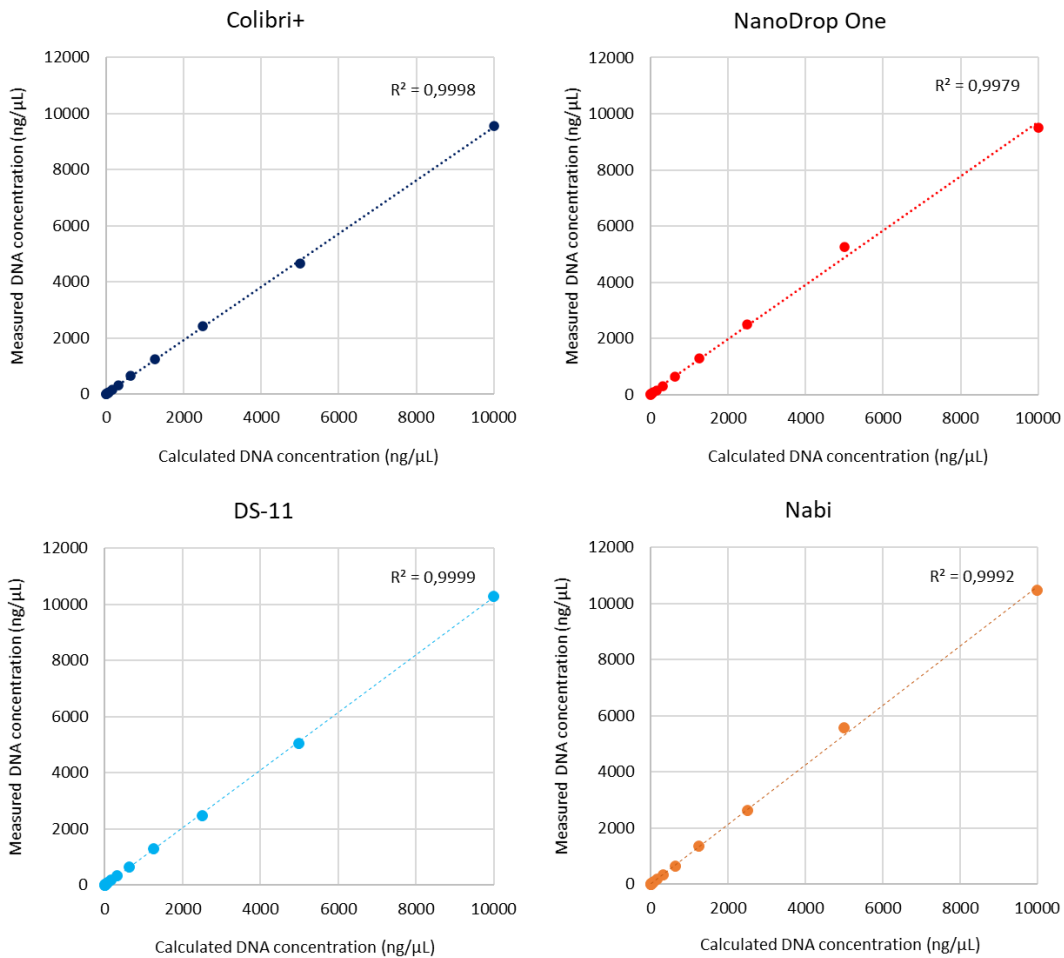


Figure 1. Linearity in the whole concentration range. All measurements in triplicate.

Figure 1 shows the linearity of all instruments in the whole concentration range tested (2.5-10,000 ng/ μ L dsDNA). All instruments displayed excellent linearity, with the DS-11 and the Colibri+ obtaining

the best correlation scores ($R^2 = 0.9999$ and 0.9998 respectively).

Conclusions

The Colibri+ has excellent accuracy, reproducibility, and linearity. Generally speaking, it has a performance comparable to that of the NanoDrop™ One and the DS-11 and has better accuracy in some concentration ranges. It has also better performance than the Nabi.

The high performance of the Colibri+, combined with fast measurement speeds (<3 s per sample), broad wavelength and concentration range, and easy and reliable pipetting, make the Colibri+ an excellent microvolume spectrophotometer for most laboratories working with nucleic acids.

References

1. Huss, V.A.R., Festl, H., Schleifer, K.H. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Systematic and Applied Microbiology*, 1983; 4(2): 184-192.

Not for use in diagnostic procedures.

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