

TN 166 DeNovix

DeNovix DS-11 Series Comparison to Thermo Fisher NanoDrop™ One Spectrophotometer

TECHNICAL NOTE

Note technique 166

Comparaison de la série DeNovix DS-11 avec le spectrophotomètre Thermo Fisher NanoDrop™ One

Introduction

The DeNovix DS-11 Series instruments and the Thermo Fisher NanoDrop[™] One spectrophotometer are used for rapid quantification of nucleic acids and proteins. The DS-11 Series Spectrophotometer / Fluorometer offers best-in-class limits of detection, the broadest dynamic range, intuitive ease of use and powerful networking features that are superior to the Thermo Fisher NanoDrop[™] One spectrophotometer. This note presents a comparison of features (Table 1) and side-by-side instrument performance (Tables 2 and 3).

The DeNovix DS-11 Spectrophotometer, introduced to the market in 2013, was the first standalone, touchscreen-controlled and network-connected UV-Vis spectrophotometer in the world. In collaboration with the life science community, DeNovix has expanded the instrument's features and functionality. In 2015, a highly sensitive fluorometer was integrated into the system, creating the DS-11 Series Spectrophotometer / Fluorometer.

The patented (US9442009) DS-11 FX+ instrument features best-in-class limits of detection for microvolume absorbance ($0.75 - 37500 \text{ ng/}\mu L \text{ dsDNA}$) and fluorescence quantification ($0.5 \text{ pg/}\mu L \text{ dsDNA}$). Labs may select the model which includes all current and future research needs by combining microvolume UV-Vis, cuvette UV-Vis and fluorescence detection into one unit.

Table 1: DeNovix DS-11 Series Spectrophotometer / Fluorometer Comparison to Thermo Fisher NanoDrop™ One spectrophotometer

	DeNovix DS-11 Series	Thermo Fisher NanoDrop™ One ^c	
	Spectrophotometer / Fluorometer	Spectrophotometer	
Fluorescence Option	Yes - DS-11 FX and DS-11 FX+ (UV, Blue, Red, Green)	None	
Dynamic Range (dsDNA)	0.5 pg/μL* - 37500 ng/μL	0.2 ng/μL - 27500 ng/μL	
Protein Performance	Outstanding	Only if sample column is properly formed	
Microvolume Lower Detection Limit	dsDNA: 0.75 ng/µL, BSA: 0.04 mg/mL	dsDNA: 2.0 ng/µL, BSA: 0.06 mg/mL	
Cuvette Lower Detection Limit	dsDNA: 0.04 ng/µL, BSA: 0.002 mg/mL	dsDNA: 0.2 ng/µL, BSA: 0.006 mg/mL	
Max Concentration	dsDNA: 37500 ng/µL, BSA: 1125 mg/mL	dsDNA: 27500 ng/µL, BSA: 820 mg/mL	
Wavelength Range	190-840 nm	190-850 nm	
Absorbance Range	0.015 - 750 A	0.04 - 550 A	
Wavelength Accuracy (Absorbance)	0.5 nm	1 nm	
Absorbance Accuracy	1.5% at 0.75 AU at 260nm	3% at 0.97 AU at 302nm	
Microvolume Pathlength 0.5 to 0.02 mm		1.0 to 0.03 mm	

	DeNovix DS-11 Series	Thermo Fisher NanoDrop™ One ^c
Pathlength Verification	SmartPath™ technology with Bridge Testing™ verification	Auto adjusting
Pathlength Control	Precision screw driven by high resolution motor	Adjustment screw driven by stepper motor
Contamination Alert Software	SmartQC™ contamination alerts	Yes
Data Export	Network Drives and Printers, Email, USB, Label Printers	USB, Label Printer, Networks
Networking	Wi-Fi and Ethernet	Wi-Fi not available in all areas, Ethernet
Color Choices	Arctic White, Brazilian Blue, Tungsten Silver, Fire Red	No
Ergonomics	Optimized screen and sample pedestal location	Extended reach required

SmartPath™ Technology with Bridge Testing™ Verification

DeNovix Spectrophotometers employ proprietary algorithms and hardware that overcome previous limitations of microvolume instruments. Analysis begins at a pathlength of 0.5 mm and compresses the sample during the measurement until the optimum pathlength is reached. DS-11 Series instruments do not stretch the droplet. Compressing prevents an unbridged condition (broken column) that can lead to inaccurate results (Figure 1).

On systems that rely on stretching samples, a broken column may occur if insufficient sample volume is pipetted for a microvolume measurement, if samples have low surface tension (common for protein solutions) or if the measurement surface loses its hydrophobic property. With Bridge Testing, DeNovix instruments detect if a sample is not bridging the optical surfaces and automatically compensates to provide a correct measurement in real time. Conversely, the Thermo Fisher NanoDrop[™] One spectrophotometer stretches the sample column to 1.0 mm and only alerts the user to a sample column anomaly without reporting a measurement result.

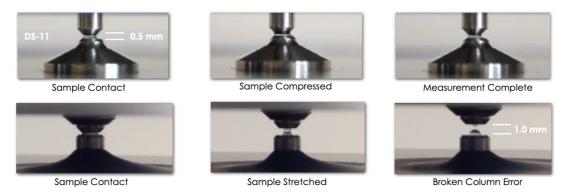


Figure 1: Top – DS-11 Series showing compression of sample droplet. Bottom – Competitor instrument showing sample stretching and breaking of the sample column.

DeNovix dsDNA Fluorescence Assays

DeNovix manufactures three ranges of highly sensitive and selective dsDNA assays. These assays, when combined with the DS-11 Series Fluorometer, enable quantification of dsDNA to 0.5 $pg/\mu L$, which is 1000x greater sensitivity than microvolume spectrophotometers. Each assay is a simple two-point standard, mix-and-measure kit. The assays allow for accurate quantification of dsDNA in the presence of RNA, degraded DNA and other contaminants.

Microvolume Performance Evaluation Methods

A 40 mg/mL solution of dsDNA was gravimetrically prepared using biotechnology grade fish sperm DNA sodium salt (Amresco cat #1B1509-256) and HPLC grade water (Ricca cat #9153-1). A series of dilutions from 12500 to 0.75 ng/µL was then prepared in HPLC grade water.

Reference concentrations for the dilutions were determined using an Agilent 8453 (Agilent, Santa Clara CA) in a 1 mm quartz cuvette (Starna, cat #1-Q-1). The reference value for DNA solutions with absorbance values outside of the Agilent's upper range of 2.0 A (equivalent to 1000 ng/µL dsDNA) were determined by gravimetrically diluting these samples to fall within the linear range of the reference spectrophotometer. Measurements were made using the dsDNA app for each instrument.

Due to the challenges of creating high concentration dsDNA samples (600-750 A), performance was evaluated using nicotinic acid and the Custom Formula Methods app on the DS-11. An 18.5 mg/mL solution of nicotinic acid was prepared gravimetrically using 99.5% Nicotinic Acid (Sigma cat #72309-100) and 0.1 N HCI (Fisher cat #SA50-1) (Ricca cat #9153-1). A series of dilutions was prepared from 18.5 to 13.5 mg/mL.

The applicable measurement app was launched on each instrument, and a microvolume mode Blank measurement was made using 1 μ L of HPLC grade water. Five sample measurements were then made for each concentration. Fresh 1 μ L aliquots were used for each replicate measurement. The sample solution was removed after each measurement by wiping the upper and lower sample surfaces with a clean, dry laboratory wipe. The

Fluorescence Performance Evaluation Methods

The DS-11 FX+ Fluoro dsDNA app and DeNovix dsDNA Quantification Assays were used in the quantification of dsDNA for this evaluation. Each assay was prepared as described in the manufacturer's protocol. Samples were mixed and incubated at room temperature for five minutes. Three replicate measurements were taken for each sample. DeNovix Assays were measured on the DeNovix Fluorometer (Table 3).

DeNovix Broad Range and High Sensitivity Assays

A series of dilutions of calf thymus DNA was prepared in TE buffer. Working solution (190 μ L) was added to a thin-walled, clear UV-transparent 0.5 mL PCR tube (DeNovix cat #TUBE-PCR-0.5-500). dsDNA (10 μ L) was added to each tube in the standard range, and volume was adjusted for total mass in the extended range.

Ultra High Sensitivity Assay

Working solution (200 µL) was added to a thin-walled, clear UV-transparent 0.5 mL PCR tube. dsDNA (10 µL) was added to each tube.

Table 2: Microvolume Performance Data

Expected	DeNovix pected DS-11 Spectrophotometer		Thermo Fisher NanoDrop™ One Spectrophotometer			
ng/µL	ng/µL	% error	%CV	ng/µL	% error	%CV
0.97	0.70	27.5	42.23	Below Specificatio	n Limits	
2.00	2.80	40.0	8.29	3.08	54.0	13.26
4.05	4.88	20.5	8.82	4.84	19.5	10.33
6.05	5.64	6.78	9.64	7.52	24.30	54.12
7.38	6.83	7.45	6.65	6.78	8.13	3.69
13.49	14.21	5.34	4.18	13.26	1.70	0.85
26.74	27.66	3.44	4.28	26.72	0.07	0.78
50.23	53.31	6.13	1.35	53.56	6.63	0.54
108.26	113.0	4.38	0.35	112.8	4.19	0.53
255.23	259.9	1.83	0.60	263.4	3.20	0.15
513.26	509.2	0.79	0.35	521.6	1.62	0.09
1036.8	1020.7	1.55	0.14	1070.2	3.22	0.22
2082.8	2102.1	0.93	0.17	2113.7	1.48	0.13
4720.8	4628.4	1.96	0.37	4524.3	4.16	0.11
10390	10118	2.62	0.67	9997	3.78	0.12
12616	12444	1.36	0.49	12042	4.55	0.26
26269	24295	7.51	0.79	23773	9.50	0.14

Table 3: Fluorescence Performance Data

Expected	DeNovix DS-11 FX+ Spectrophotor	neter / Fluorometer	Thermo Fisher NanoDrop™ One Spectrophotometer	
ng/µL	ng/µL	%CV	Assay*	
0.0005	0.00036	6.89	UHS	Fluorescence quantitation not possible.
0.0010	0.00109	8.75	UHS	Requires purchase of additional instrument.
0.0020	0.0018	2.23	UHS	
0.0100	0.0085	1.06	UHS	
0.0500	0.0452	0.40	UHS	
0.10	0.07	0.00	HS	
0.30	0.25	0.16	HS	

Expected	DeNovix DS-11 FX+ Spectrophotor	neter / Fluorometer	Thermo Fisher NanoDrop™ One Spectrophotometer	
1.00	0.79	0.10	HS	
3.00	3.04	0.20	HS	
10.0	10.6	0.36	HS	
25.0	26.8	0.11	BR	
50.0	53.8	0.10	BR	
100	106	0.09	BR	
200	196	0.14	BR	
1000	1050	0.01	BR	

Table 4: High Absorbance Sample Performance

Expected	dsDNA Equivalent	DeNovix DS-11 Spectrophotometer		tometer	Thermo Fisher NanoDrop™ One Spectrophotometer
Abs	ng/µL	Abs	% error	%CV	Abs
600	30000	588.8	1.87	0.35	Above Specification Limits
650	32500	628.9	3.24	0.41	
700	35000	676.7	3.36	0.66	
750	37500	732.7	2.44	1.32	

Summary

The DeNovix DS-11 Series Spectrophotometer / Fluorometer delivers greater accuracy and sensitivity than the Thermo Fisher Scientific NanoDrop™ One spectrophotometer. The DeNovix DS-11 also offers a broader dynamic range, greater ease of use and provides researchers with superior flexibility and functionality.

The DS-11 Series Spectrophotometer / Fluorometer combines microvolume absorbance and fluorescence measurements on one instrument, supplying the user with tools to measure both routine and difficult samples. With the DS-11 Series, labs are equipped for today's complex workflow as well as for the challenges of future assays. The DS-11 Series and DeNovix Assays combined provide superior sensitivity and a much broader dynamic range for dsDNA quantification (0.5 $pg/\mu L$ – 37500 $ng/\mu L$) than the Thermo Fisher NanoDropTM One spectrophotometer.

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DeNovix Inc. 3411 Silverside Road Wilmington, DE 19810, USA Phone: +1.302-442-6911 Email: info@denovix.com www.denovix.com

