BioDrop Duo+ dsDNA Application Note

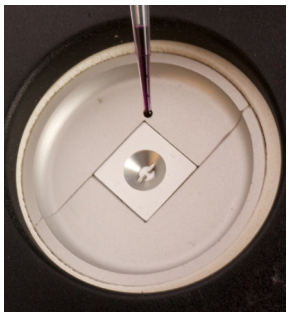
BioDrop
a division of Harvard Bioscience, Inc.

Using a BioDrop Duo+ spectrophotometer to measure the concentration of low volume samples of dsDNA

Micro-volume measurement of DNA is a routine application in many life science laboratories. Quantification and purity measurement of dsDNA is a key first step before performing experiments such as PCR, qPCR, Next Generation Sequencing, ChIP and ChIP Seq. The success of these experiments requires accurate and precise quantification of the dsDNA starting material. In addition, the high cost of the reagents makes accurate initial quantification even more important. Life scientists also require the flexibility of working with higher concentrations offered by the BioDrop CUVETTE 125 and larger volumes used in conventional spectrophotometry.

Current options for direct measurement of dsDNA are conventional spectrophotometers or dedicated micro-volume instruments. Conventional UV/Vis Spectroscopy requires sample dilution, adding time to the quantification method, as well as increasing the possibility of error due to additional sample manipulation. Existing micro-volume instruments have multiple disadvantages such as possible pathlength drift and inaccuracy in detection limits as well as issues such contamination and bubbles disrupting analysis.

To address these issues, BioDrop developed a novel micro-volume instrument, the BioDrop Duo+. The BioDrop Duo+ is a UV/Vis spectrophotometer which has a novel in-built sample port with a fixed 0.5 mm pathlength AND a cuvette holder to accommodate the BioDrop CUVETTE or a standard 10 mm cuvette. The sample port improves the scientist's ability to make measurements of highly concentrated and/or low volume DNA, RNA, oligonucleotides and protein samples, whilst the option of BioDrop CUVETTE analysis provides life scientists the flexibility to measure more concentrated samples. Because the BioDrop Duo+ has a fixed pathlength, excellent measurements can be achieved without the need to calibrate. Concentrations are obtained quickly using a colour touchscreen to select pre-programmed methods in the on-board software. Importantly, users do not need to perform time-consuming sample dilution.



A key application of direct micro-volume measurement is the quantification of dsDNA. To demonstrate the performance of the BioDrop Duo+, a series of experiments were performed to determine the minimum sample volume, detection limit, reproducibility, linearity and sample carry over. For a summary of results, see Table 1.

Table 1. Summary of Results

	Sample Port	BioDrop CUVETTE 125
DNA Detection Limit (ng/µL)	1.0	7.1
Minimum Volume (µL)	0.5	0.6
Maximum Concentration (ng/µL)	2,500	10,000
Pathlength (mm)	0.5	0.125

Materials and Methods

The BioDrop Duo+ is a split-beam, UV/Vis spectrophotometer which uses a Xenon light source and a 1024 CCD array detector. This technology delivers rapid, high quality measurements. Details of the technical specifications can be found in Table 2.

The BioDrop Duo+ generates quick results: no warm-up time is required and the instrument is readily controlled using a high-resolution colour touchscreen. Samples are pipetted directly into the in-built sample port or the cuvette, and then measured.

Materials and Methods

Purified Salmon Sperm dsNA (Sigma-Aldrich Item # D1626-250MG) was dissolved in HyPure Molecular Biology Grade H2O directly before measurement using the BioDrop Duo+ Spectrophotometer.

The 0.5 mm pathlength was selected from the drop-down menu of the on-board software for measurements using the in-built sample port of the BioDrop Duo+. Alternatively a 0.125 mm pathlength was selected for analysis using the BioDrop 125 CUVETTE. DNA concentrations were calculated automatically using the pre-programmed DNA quantification method: absorbance is measured over a wavescan from 230 to 320 nm. The concentration of dsDNA in the solution was determined by subtracting the absorbance at 320 nm from absorbance at 260 nm and then multiplying the corrected absorbance by the concentration factor of DNA (50 µg/mL) and the pathlength normalisation factor (20 (in-built sample port) or 80 (BioDrop CUVETTE 125)).

Multiple instruments were tested to determine the detection limit, minimum sample volume, linearity, reproducibility and carry over. Data representative of the test group is shown in Figures 1-3.

The detection limit was determined by performing a series of twenty measurements in the in-built sample port using the DNA method on the on-board software.

Minimum sample volume was assessed by performing a series of measurements using a 1 µL microsyringe. The reproducibility of the measurement at decreasing volume was determined by calculating the mean and standard deviation.

The linearity of the instrument was determined by plotting the measured concentration against dilution of the sample.

Finally, sample carry over was assessed by alternating measurements of water and 100 and 1000 ng/mL dsDNA solutions. The in-built sample port was simply wiped with lint-free tissue in between measurements to evaluate the effectiveness of cleaning between samples.

Table 2. Technical Specifications

Display	7” colour capacitive touchscreen
Light Source	Pulsed Xenon lamp with 3 year warranty
Detector	1024 element CCD array
Wavelength Range	190 nm to 1100 nm
Pathlength Accuracy	In-built sampling port has an accuracy of +/- 5 µm
Wavelength Accuracy	± 2 nm
Wavelength Reproducibility	± 1 nm
Spectral Bandwidth	5 nm
Stray Light	<0.5%T at 220 nm NaI and <0.5%T 340 nm using $NaNO_2$
Absorbance Range	- 0.3 A to 2.5 A, 0 to 199%T
Absorbance Accuracy	±0.005 A or 1% of the reading, whichever is the greater at 546 nm
Absorbance Reproducibility	±0.003 A (0 to 0.5 A), ±0.007 A (0.5 to 1.0 A)
Noise	0.005 A peak to peak 0.002 A RMS
Output	USB port for USB memory stick
Power input	120 to 240V,~ 50/60Hz 40VA Max
Dimensions	Height 190 mm x Width 280 mm x Depth 410 mm
Weight	Approx. 3.55kg
Life Science PC Software	DNA, RNA, Oligo, Fluorescent Dye, Tm Calculation, OD600, Protein Dye, Protein UV and Colorimetric protein methods
Applications	Single Wavelength, Concentration, Wavescan, Kinetics, Standard Curve, Multi-wavelength, Substrate, Equation Editor
Software Languages	English, French, German, Italian, Japanese, Spanish & Simplified Chinese

Results

Linearity of Measurement of the BioDrop Duo+

A range of DNA concentrations were measured. Each sample concentration was measured five times. The mean was calculated for each concentration and then plotted against the dilution factor. The BioDrop Duo+ demonstrated excellent linearity of up to 2500 ng/µL as demonstrated by an R2 value of 0.9998 as shown in Figure 1. The R2 value refers to the accuracy of measurement within a linear line, in which measurements displaying clear linearity exhibit an R2 value of 0.9 to 1.0.

The linearity of the BioDrop CUVETTE 125 was also measured in the BioDrop Duo+. Measured concentrations were plotted against the dilution factor. Excellent linearity was achieved as demonstrated by an R2 value of 0.9997.

dsDNA Detection Limit of the In-Built Sample Port

The dsDNA detection limit of the BioDrop Duo+ in-built sample port is an important specification which informs the user of the minimum concentration that can be measured. A low detection limit allows end users to use the minimal amount of sample for measurement thereby allowing for more sample to be used for subsequent experiments. The detection limit of the micro-volume sample port shown repeatedly to be less than 1 ng/µL, a best in class specification, as shown in figure 3. The detection limit when using the BioDrop CUVETTE 125 was 7 ng/µL.

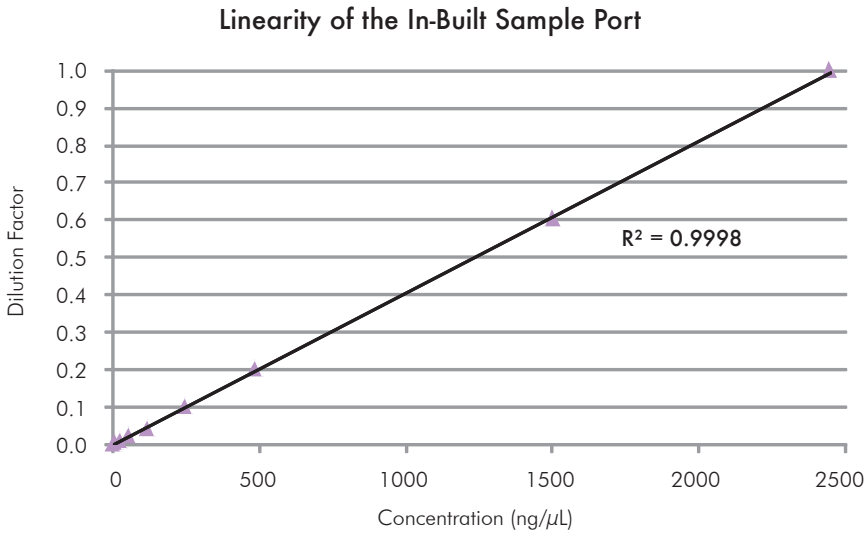


Figure 1: Linearity of the in-built sample port was determined by plotting concentration against the dilution factor.

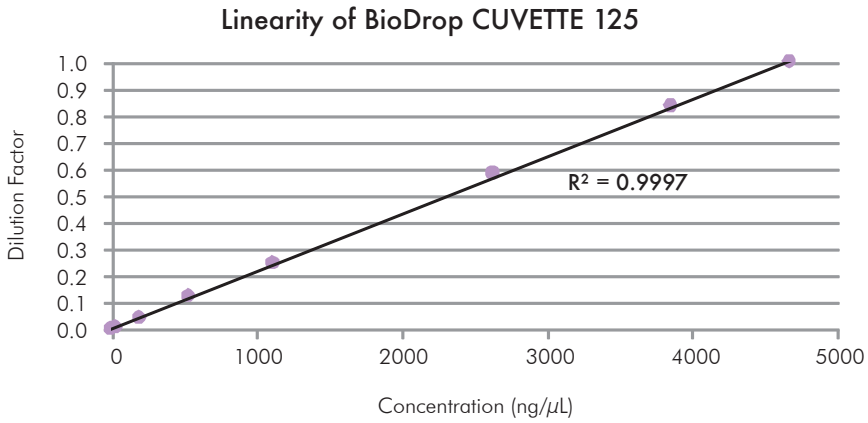


Figure 2: Linearity of the BioDrop CUVETTE 125 was determined by plotting concentration against dilution factor.

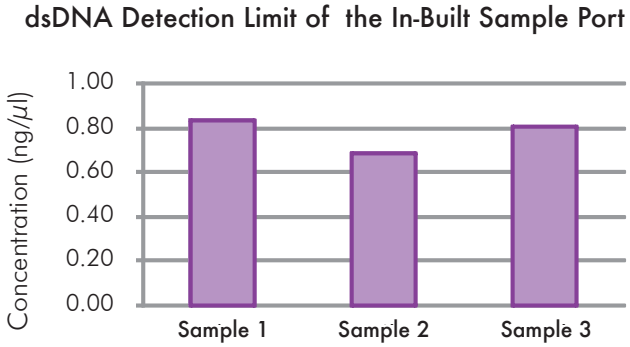


Figure 3: Detection limit of three independent in-built sample ports were analysed.

Reproducibility and Minimal Carry Over Between Samples

Measurements were made to determine if samples are carried over from one measurement to the next. A simple wipe with a lint-free tissue is sufficient to reduce sample carry over to non-detectable amounts as shown in Figure 4. The BioDrop CUVETTE 125 was examined for DNA carry over in Figure 5.

Minimum Volume Sampling

Another key measurement for micro-volume quantification of dsDNA, is the lowest volume that can be pipetted and measured to achieve reproducible and accurate results. For this purpose, we used a 1 µl microsyringe to pipette 1000 ng/µL. Figure 5 displays the minimum volume pipetted onto the built-in sample port of the BioDrop Duo+ which is 0.5 µl, which is ideal for sample preservation.

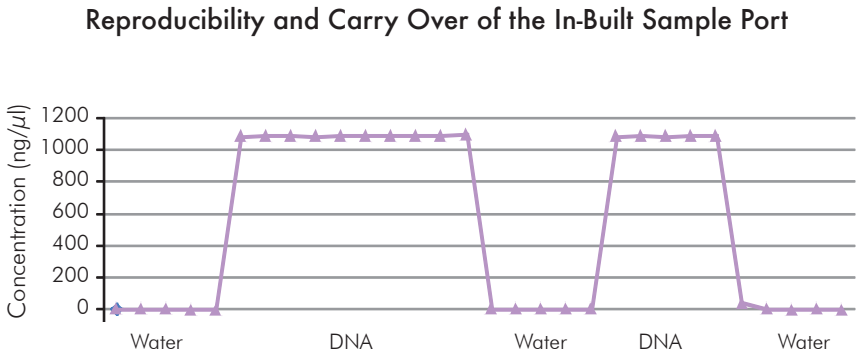


Figure 4: DNA carry over was analysed by measuring sample concentrations (via the in-built sample port), which were immediately followed by water.

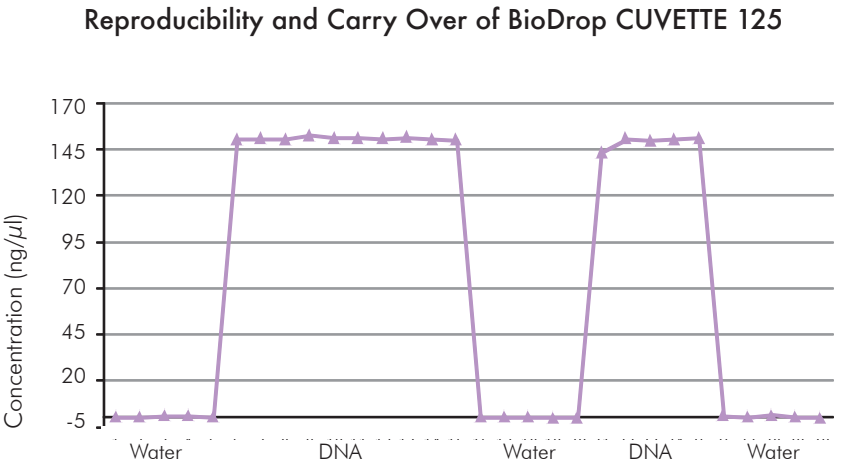


Figure 5: DNA carry over was analysed by measuring sample concentrations (via the BioDrop CUVETTE 125), which were immediately followed by water.

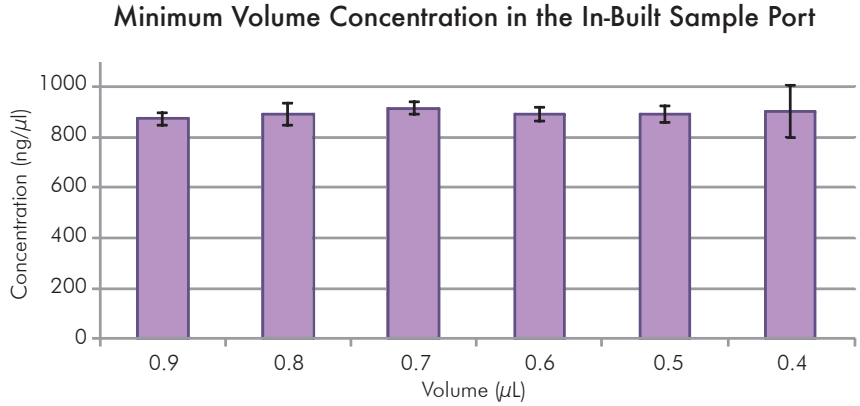


Figure 6: Minimum volume measurements of >1µl were measured to assess reproducibility at low volumes

Typical Methods Requiring Micro-volume Measurements

Table 3 highlights the key downstream applications for nucleic acids following quantification. These assays require high sample concentration and low volumes.

BioDrop Resolution Software

BioDrop Resolution Life Science PC software is a powerful yet intuitive software package that is included with the BioDrop Duo+. BioDrop Resolution Life Science software allows rapid and efficient measurements as well as analysis. The software contains methods for DNA, RNA, oligonucleotides and protein analysis. Table 4 summarises the key features of BioDrop Resolution Software.

BioDrop also provides an optional CFR module for laboratories requiring full 21 CFR part 11 compliance. Features included in this version allow restricted access for specified groups and administration rights.

Table 3. Key Downstream Molecular Assays Requiring DNA quantification

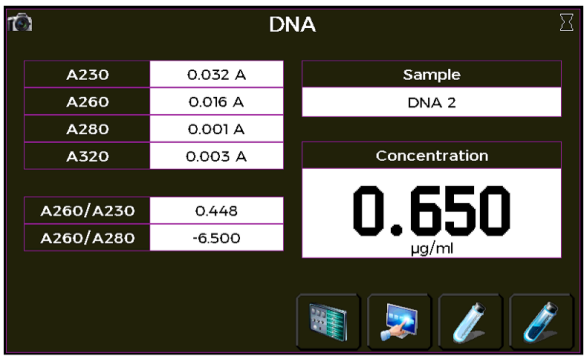
CHIP Sequence	10 ng / 30 µl (0.3 ng/mL)
Sequencing	125 ng / 20 µl (6 ng/mL)
Next Generation Sequencing	10 ng/µl
Transfections	5 to 30 µg / 100 µl
DNA Vaccines	0.5 to 2 mg
PCR	2 ng/µl
qPCR	200 ng / 100 µl
DNA Microarray	>2 µg
siRNA	7.5 µg/mL
Chromosomal Capture Conformation	2 to 3 ng/ µl

Table 4. BioDrop Resolution Software

Life Science Features	Concentration and purity measurements of DNA, RNA, oligonucletoides, and proteins
Method Developer	Design your own experimental protocol. Tailor your analysis to suit your requirements.
Quick Read	Measure the absorbance of unknown samples prior to further experimentation.
Quick Scan	Measure a wavelength scan of samples of unknown absorbances to confirm peak wavelengths.
Cy dye analysis	Absorbance measurements to assess the labelling efficiency of DNA and/or protein samples.

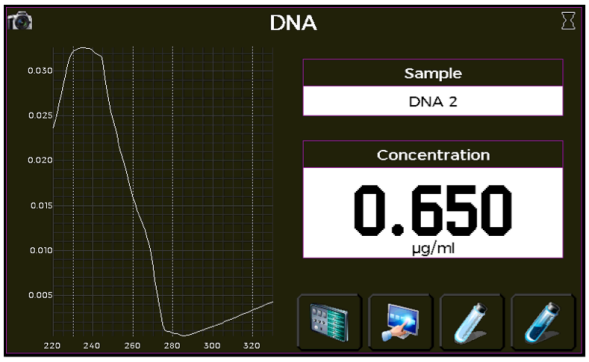
On-Board Software

BioDrop Duo+ can be controlled via a colour high-resolution touchscreen for stand-alone analysis. The on-board software is both user friendly and easy to navigate. The software provides both quantitative and qualitative data analysis making analysis as well as measurement both simple and efficient.



The method for the analysis of DNA uses a wavelength scan for both quantification and purity. Four absorbance measurements are shown:

1. A230 nm– Absorbance peak of possible sample preparation contaminants like phenol and chloroform.
2. A260 nm– DNA absorbance peak.
3. A280 nm– Protein absorbance peak.
4. A320 nm– No molecular absorbance, used as a background measurement.



Conclusion

The BioDrop Duo+ is a dedicated micro-volume instrument which represents a clear improvement over existing instrumentation. Using no moving parts, the BioDrop Duo+ uses an in-built sample port to deliver an unrivalled dsDNA detection limit of 1 ng/µl. A maximum concentration of dsDNA can be achieved of 2500 ng/µL. Using the BioDrop CUVETTE 125 in the BioDrop Duo+ extends the measurement range up to 10,000 ng/µL of dsDNA whilst only using 0.6 µL of sample. The in-built sample port is easy to use and sample volumes as low as 0.5 µL can be pipetted and measured accurately. Importantly, the in-built sample port is easy to clean thereby preventing sample carry over. Ultimately the BioDrop Duo+ is easy to use with excellent specifications, superior ease of use and powerful features. The BioDrop Duo+ delivers a new standard of excellence in micro-volume measurement and you never need to calibrate.