Measurement of Ultra Micro Volumes of Nucleic Acids Using the PerkinElmer LAMBDA Bio+ and the Hellma® TrayCell™

Figure 1. LAMBDA Bio+ with Hellma® TrayCell™ with 600 ng/mL (approx.) sample.

Introduction

The need to measure ultra micro (<5 µL) volumes of nucleic acid is common in molecular biology. Typical applications include the quantitation of template prior to sequencing, PCR quantitation and purity analysis ("260/280 ratio"). While larger volumes can be readily measured in micro-, semi-micro or full size cuvettes, handling ultra micro amounts in very low volume ultra microcells has presented particular practical challenges. The difficulties presented with such small amounts of material include accuracy of pipetting, carryover and the issue of electrostatic interaction between droplets of the liquid and the plastic pipette tips.

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The PerkinElmer® LAMBDA™ Bio and LAMBDA Bio+ are designed for the measurement of nucleic acids and proteins (the LAMBDA Bio+ can also be used as a general purpose spectrophotometer). These are generally used with microcells (e.g. 50 µL) or larger volumes but they give excellent results when used with the Hellma® Tray Cell. This combination results in a very flexible system which is able to measure sample amounts from 3 µL to 3 mL. The TrayCell™ is extremely easy to use and carryover is eliminated due to its inherent design. The instrument is self-contained and so an external PC is not required. Data can, however, be transferred easily to a PC from the LAMBDA Bio+ via the USB link cable and software supplied as standard with the instrument.*

TrayCell™

The TrayCell™ is a 1 cm x 1 cm device that fits into the standard cuvette position on the spectrophotometer. The device is offered in a variety of beam heights but 15 mm should be used for all PerkinElmer UV/Vis spectrophotometers. Inside, the unit has a system of prisms and fiber optics which serve to periscope the light beam up to the sampling window and then back to the detector on the instrument (see Figure 2). The device is supplied with a cap which contains a small mirror. This cap ensures that the sample is measured at constant pathlength. The pathlength is achieved by a double pass through the sample as controlled by the cap height. For the examples described in this technical note, a pathlength of 0.02 mm was used. The instrument's internal software is able to adjust this value to the equivalent for a 1 cm cell by applying a factor of 50 to all measurements. There is no alignment procedure when the TrayCell™ is used with the LAMBDA Bio+ and the device remains in the instrument during all phases of the measurement including filling (see Figure 3). The user simply pipettes about 3 µL of liquid onto the window and then places the cap on top. The measurement can then be taken.

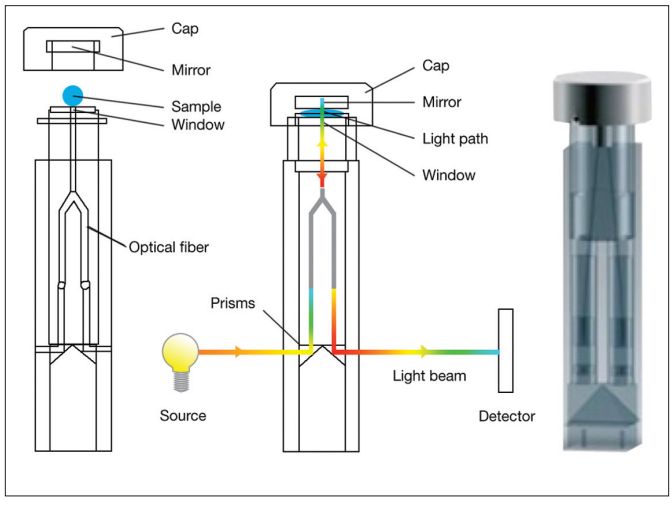

Figure 2. Configuration of the Hellma®.

Experimental

Two samples of calf thymus DNA with respective concentrations of 600 and 1300 ng/µL (equivalent to mg/L) were prepared and measured using the LAMBDA Bio+ fitted with the Hellma® TrayCell™. A 3 µL drop was deposited onto the quartz window on the TrayCell™ so as to cover it. The cap was then replaced and the measurement taken. All samples were blanked using deionized water.

Results and discussion

Samples were scanned and the data transferred to a PC using the software and USB cable supplied with the LAMBDA Bio+ (see Figure 4). The purity and concentration were also measured using the DNA measurement method, and a concentration factor of 50 was applied for double-stranded DNA (dsDNA). For single stranded DNA, this factor should be 33 and for RNA 40. The purity was measured at 260, 280 and 320 nm (Table 1). The 320 nm absorbance value was subtracted from the respective 260 nm and 280 nm measurements and then a ratio calculated. For the concentration measurement, the 320 nm absorbance value was subtracted from the 260 nm reading and then multiplied by the concentration factor. The pathlength compensation factor (50) was applied in order to adjust values to a 1 cm cuvette. A purity ratio of over 1.7 indicates that the DNA is pure and not contaminated with protein.

The results were checked against a LAMBDA 25 dual beam UV/Vis spectrophotometer and found to be in good agreement.

Figure 3. Close-up TrayCell™ in the LAMBDA Bio+.

*Can be purchased separately for standard LAMBDA Bio.

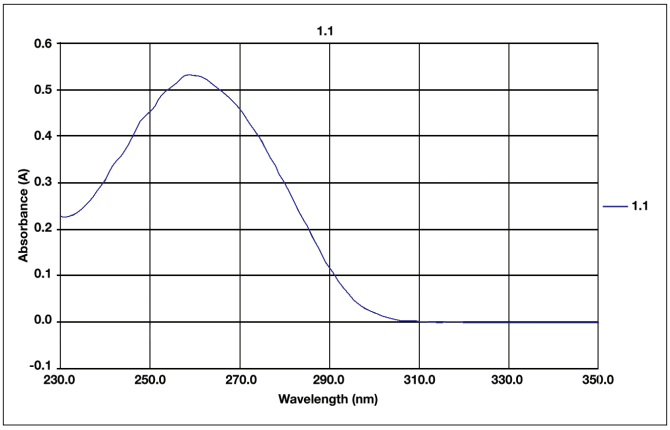

Figure 4. 500 ng/µL calf thymus DNA plotted in MS Excel® format.

Table 1. Example of results output from LAMBDA Bio/Bio+.

Concentration: 610 ng/µL			
A230	A260	A280	A320
0.105	0.246	0.141	0.002
A260/A280	A260/A230		
1.755	2.369		
Concentration: 1292 ng/µL			
A230	A260	A280	A320
0.212	0.515	0.289	-0.002
A260/A280	A260/A230		
1.777	2.416		

Conclusion

The Hellma® TrayCell™ has been shown to be an ideal companion to the LAMBDA Bio/Bio+ spectrometer for the measurement of ultra low volumes of liquids such as Nucleic acids and proteins.

Ordering information

L7110289	TrayCell™ Accessory including 0.2 Cap and 1.0 mm Cap
L7110290	TrayCell™ 2.0 mm (Factor 5) Cap
L7110291	TrayCell™ 0.2 mm (Factor 50) Cap
L7110292	TrayCell™ 0.1 mm (Factor 100) Cap
L7110293	TrayCell™ 2.0 mm (Factor 5) Cap
L7110287	LAMBDA Bio UV/Vis Spectrometer with TrayCell™
L7110288	LAMBDA Bio+ UV/Vis Spectrometer with TrayCell™

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