GENOVA Application note: A09-003A

DNA measurements using the TrayCell

Introduction

One of the most common applications of a UV/Visible spectrophotometer is to measure the concentration of nucleic acids. Often there is very little sample available and the researcher may not wish to dilute it further in order give sufficient volume for measurement in a standard quartz cuvette. There are ultra-micro quartz cuvettes available which will allow as little as 20µl of sample to be measured directly, however they can be quite difficult to use to achieve reproducible results. An alternative, if the sample is of a sufficient concentration, is to use a TrayCell.

The TrayCell is a fibre-optic, ultra-micro cell designed for measurements of extremely small sample volumes of DNA, RNA or protein. The dimensions of the TrayCell are equivalent to a standard cuvette and so will fit in all Jenway spectrophotometers. The TrayCell is extremely easy to use. It is first positioned in the cell holder of the spectrophotometer and the sample is then placed on an optical window on the surface of the cell. A cap containing a mirror is then fitted over the top. The type of cap used determines the path length through which the light passes.

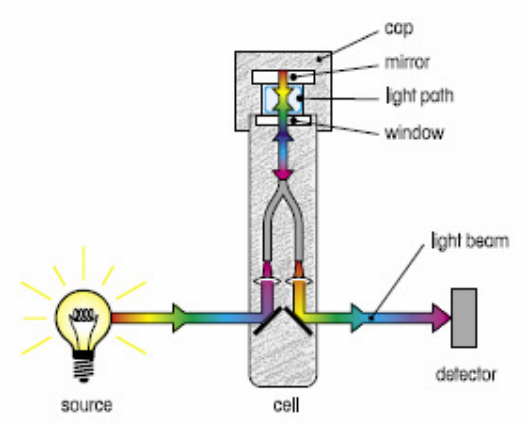

Figure 1: Schematic diagram of the light path in the TrayCell.

Light from the spectrophotometer lamp is directed to the sample in the cap by optical fibres; the light returned by the sample is reflected off the mirror in the cap and passed down a second optical fibre to the detector. Once the measurement has been made, the cap is removed and the sample can be recovered if required. The window and cap are then gently cleaned using a lint-free swap or wipe. The TrayCell remains in the cell holder during all stages; this ensures that the aperture remains in an identical position for each measurement for increased reproducibility.

There are two caps supplied with the TrayCell. Using the 1mm or 0.2mm cap creates a defined optical light path of 1mm and 0.2mm respectively. This generates virtual dilution factors of 1:10 or 1:50 in comparison to a measurement with a standard 10mm cuvette. This means that a sample giving an absorbance reading of 1.0 in a 10mm path length cuvette will give an absorbance of 0.1 using the 1mm cap and 0.02 with the 0.2mm cap. Therefore it is important that the sample to be measured using the TrayCell is sufficiently concentrated to give a reading of at least 0.05 absorbance units for reproducible measurements.

In this application note we demonstrate DNA concentration measurements using the TrayCell in the Jenway Genova and compare the results with those from other commonly used cuvettes.

Methods

A 1mg/ml solution of genomic DNA was diluted with 10mM Tris, 1mM EDTA solution, pH 8.0 (TE buffer) to give a range of concentrations down to 1µg/ml. Samples of these dilutions were measured, as appropriate, in the cuvettes listed in Table 1 using the recommended fill volumes for each cuvette.

Description	Jenway part code	Recommended fill volume
UV/Vis plastic cuvette	035 143	70µl
Quartz ultra-micro cuvette	035 138	100µl
Quartz ultra-micro cuvette	035 249	20µl
TrayCell 1mm cap	JEN/105-810-UVS	5µl
TrayCell 0.2mm cap	JEN/105-810-UVS	2µl

Table 1: Cuvettes used for this comparison.

Where 035 143 was used, the Genova was fitted with the micro-cuvette holder (630 304) to prevent excess light scattering through the side walls of the cuvette.

Measurements in the Genova were made using the 260/280nm mode with correction (selected as YES in the SETUP menu) at 320nm. The SETUP menu also allows entry of sample dilution. This was used to correct for the "virtual" dilution factors of the TrayCell caps. For the 10mm path length cuvettes, the dilution was set to 0001+0000µl (no dilution); for the 1mm cap it was set to 0001+0009µl (1 in 10 dilution) and for the 0.2mm cap it was set to 0001+0049µl (1 in 50 dilution). Concentrations were recorded as µg/ml.

Results

DNA concentrations for each dilution were calculated by the Genova software based on the default factor values and virtual dilution factor of the sample. The equation used by the Genova is[1]:

$$\text{Concentration} = ((Abs_{\lambda 1} - Abs_{REF}) \times Factor_1 - (Abs_{\lambda 2} - Abs_{REF}) \times Factor_2) \times Dilution$$

Where:
$Abs_{\lambda 1} = Abs_{260}$; $Abs_{REF} = Abs_{320}$; $Abs_{\lambda 2} = Abs_{280}$;
Factor 1 = 62.9; Factor 2 = 26;
Dilution = 1, 10 or 50 depending on cuvette.

The values obtained from each cuvette are displayed in Figure 2 and summarised in Table 2.

	DNA concentration (µg/ml)				
Sample conc. (µg/ml)	035 143	035 138	035 249	Tray Cell 1mm	Tray Cell 0.2mm
1	0.60	0.69	-0.33		
5	4.74	4.40	5.19	4.91	
10	9.02	9.32	9.23	11.13	
25	22.55	22.53	21.35	24.28	
50	44.75	45.11	40.8	45.47	41.77
100	85.30	87.39	79.62	90.95	94.18
125				114.8	116.0
250				226.6	244.6
500				480.4	501.6
1000				922.1	1034

Table 2: Measured DNA concentrations of each sample dilution in various cuvettes.

The results demonstrate that the TrayCell with either cap gives comparable values to the samples measured in conventional 10mm path length cuvettes. Due to the virtual dilution factor of the caps, it is possible to measure DNA of a much higher concentration (greater than 125µg/ml) without diluting the sample.

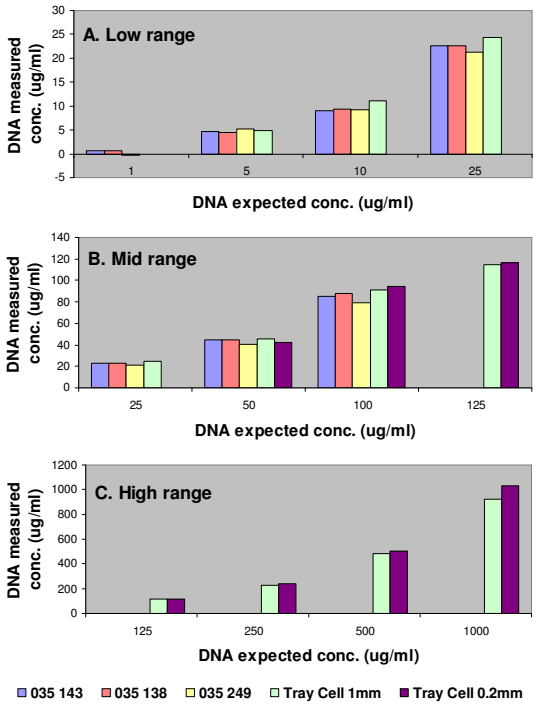

Figure 2: DNA concentrations of each dilution as measured in various cuvettes.

At lower concentrations, the theoretical limits of the TrayCell for dsDNA (based on an absorbance at 260nm of 1 for a 50µg/ml solution and the lowest accurate reading of 0.05 Abs) are 25µg/ml and 125µg/ml for the 1mm and 0.2mm caps respectively. Although Table 2 shows that it was possible to obtain results with lower concentrations than these, it should be noted that the actual absorbance values at 260nm were below 0.05 and the values at 280nm were also too low to give accurate A_{260}/A_{280} ratios in the normal, expected range of 1.7-2.0 (Table 3).

	A_{260}/A_{280} ratios				
Sample conc. (µg/ml)	035 143	035 138	035 249	Tray Cell 1mm	Tray Cell 0.2mm
1	-1.64	1.15	0.29		
5	1.95	1.72	2.71	-0.17	
10	1.98	1.7	2.32	3.33	
25	1.93	1.81	2.15	3.32	
50	1.91	1.8	2.11	1.9	1.81
100	1.86	1.8	2.32	1.92	1.78
125				1.88	1.98
250				1.84	2.11
500				1.84	1.79
1000				1.79	1.84

Table 3: A_{260}/A_{280} ratios of each sample dilution in various cuvettes.

Conclusions

The TrayCell is a useful tool for measuring the absorbance of samples where there is limited sample available or the sample needs to be recovered after measurement without dilution. The required sample volume for the 1mm cap is 3µl to 5µl and for the 0.2mm cap, 0.7µl to 4µl. Therefore if the sample is sufficiently concentrated, only a very small volume is required. Concentration values obtained with the TrayCell are comparable to those obtained with standard 10mm path length cuvettes.

A minimum concentration of 25µg/ml dsDNA is required for the 1mm cap and 125µg/ml for the 0.2mm cap. The table below summarises the requirements for other nucleic acids.

	Sample specific factor	1mm cap (µg/ml)	0.2mm cap (µg/ml)
dsDNA	50	25-850	125-4,250
ssDNA	37	18-630	90-3,150
ssRNA	40	20-680	100-3,400
Oligo	30	15-510	75-2,550

Table 4: Range of nucleic acid detection for the TrayCell with 1mm or 0.2mm caps. Sample specific factor refers to the concentration in µg/ml which gives an absorbance of 1 at 260nm in a 10mm path length.

Applications where the TrayCell might be used could include:

1. Measurement of DNA stocks/standards for concentration verification.
2. Measurement of oligonucleotides or PCR primers after reconstitution.
3. Measurement of plasmid DNA concentration after mini-prep extraction.
4. Other applications where absorbance levels are too high to be read in conventional 10mm path length cuvettes.

References

1. O. Warburg, W. Christian (1941), Biochem. Z. **310**, 384 – 421.