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Application News

No. AD-0088

UV-1280, TrayCell, Nucleic Acid

Micro Volume Photometric Measurement of Nucleic Acid using TrayCell with UV-1280

❑ Introduction

Nucleic acid concentration can be determined easily using an ultraviolet-visible (UV-VIS) spectrophotometer. Such samples are typically available in small micro volume (less than 5 µl), and a system that is able to measure micro volume with high reproducibility will be preferred. A conventional 10 mm path length super micro cuvette requires at least 50 µl of sample and needs extra care in cleaning to prevent carryover. Hellma TrayCell is a fiber-optic ultra-micro cell designed for the UV-VIS micro volume analysis of nucleic acids and proteins. The sample volume requirement ranges from 0.7 µl to 10 µl depending on the path length used. Shimadzu UV-1280 offers a bio method mode as a standard function for DNA and protein concentrations determination.

❑ Bio method Mode of UV-1280

The UV-1280, Figure 1, has a bio method mode consisting of six measurement methods as shown in Figure 2. The measurement method can be selected from 1. DNA Quantitation, 2. Lowry Method, 3. BCA method, 4. CCB Method, 5. Biuret Method, and 6. UV Method.

Figure 1: UV-1280

The [1. DNA Quantitation] measurement screen is displayed in Figure 3. DNA has an absorbance peak at 260 nm and a valley at 230 nm. Protein has a peak at 280 nm. The purity of the nucleic acid in a biological sample is commonly determined by the absorbance ratio of 260 nm and 280 nm. Pure DNA samples have A_{260}/A_{280} of 1.8 to 1.9. If there is a contamination by proteins or other contaminants that absorb at or near 280 nm, the ratio will be significantly lower. Furthermore, the purity of DNA can also be judged using absorbance ratio of 260 nm and 230 nm. Pure DNA samples will have A_{260}/A_{230} of more than 2.0. If the ratio is considerably lower than expected, it may indicate the presence of contaminants, for example carbohydrates and phenol, which absorb at 230 nm.

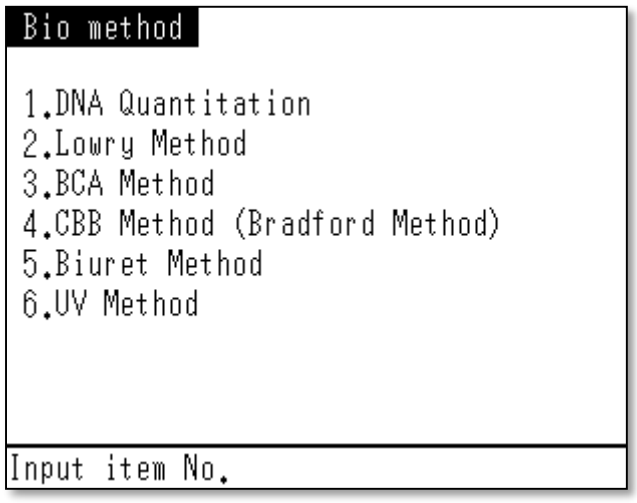

Figure 2: Bio method Selection Screen

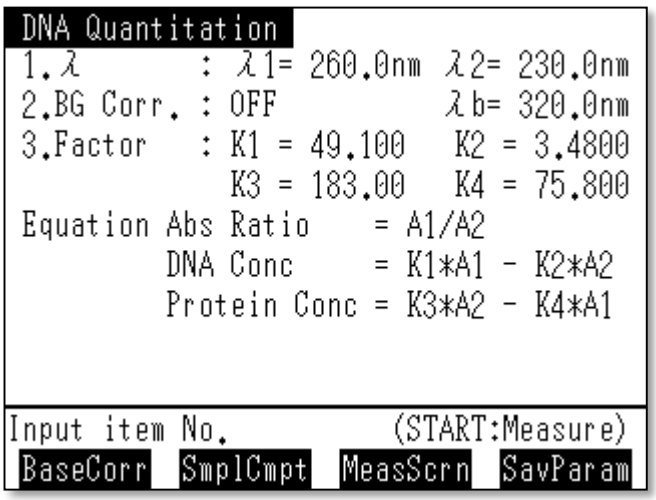

Figure 3: DNA Quantitation Screen

❑ Hellma TrayCell

The dimensions of the TrayCell from Hellma are equivalent to a standard 10 mm path length cuvette which allows it to fit into the cuvette holder of the spectrophotometer. The TrayCell consists of a fiber-optic measuring cell and a cap with an integrated mirror. Light is guided up to the sample via prisms and fiber-optic, reflected in the mirror and then guided back out of the TrayCell to the detector on the instrument, as illustrated in Figure 4. The precisely defined spacing between the cell window and the mirror in the cap ensures that the optical path length is accurate and remains constant.

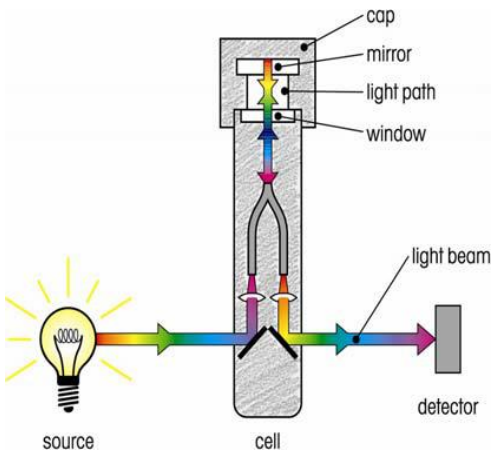

Figure 4: TrayCell Light Path

The 1 mm or 0.2 mm cap creates a defined optical light path of 1 mm and 0.2 mm respectively. This generates virtual dilution factors of 1:10 or 1:50 in comparison to a measurement with a standard 10 mm cuvette. This feature saves time and avoids dilution errors.

❑ Experimental

In this example, TrayCell with a path length of 1 mm was used with UV-1280 to measure a 100bp DNA ladder from Promega. The required sample volume is 3 µl to 5 µl. Here, an amount of 3 µl of sample was used for measurement. The reference blank was pipetted onto the cell window as shown in Figure 5 and covered with the cell cap. Baseline correction was performed to zero the absorbance reading from the reference blank and the cell. The same procedure was conducted for the sample. The sample was measured by pressing the [START] button on UV-1280.

Figure 5: Hellma TrayCell

❑ Results and Discussion

The absorbance ratio, DNA concentration and protein concentration are displayed as shown in Figure 6.

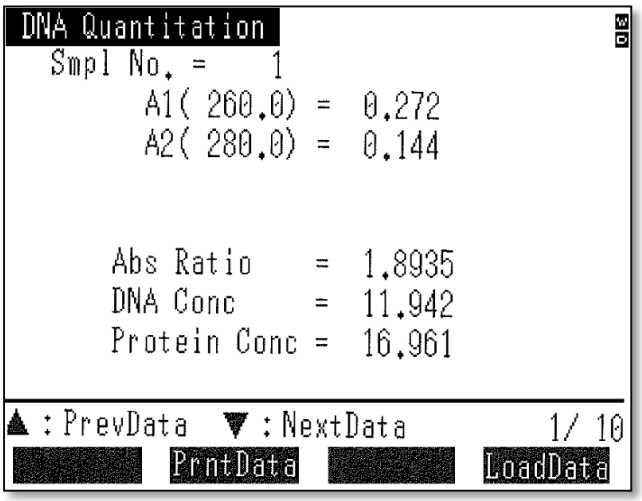

Figure 6: DNA Quantitation Result Display

Table 1. Absorbance of DNA ladder measured ten times at 260 nm

	Absorbance at 260 nm
1	0.272
2	0.277
3	0.282
4	0.274
5	0.273
6	0.273
7	0.269
8	0.278
9	0.275
10	0.279
Average	0.275
SD	0.004
RSD (%)	1.32

Table 1 shows the absorbance values, standard deviation (SD), relative standard deviation (RSD) for ten replicate measurements at 260 nm. A low SD of 0.004 and good RSD of 1.32 % were obtained.

❑ Conclusions

The Hellma Traycell together with UV-1280 Spectrophotometer bio method mode provides an easy measurement of micro volume of nucleic acids with good repeatability.

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