Application Note

UV-0017

DNA Melting Measurements (3) with the PAC-743/ 743R Water-cooled Peltier Cell Changer
"1 mm 10 µL 8-Position Micro Cell Block"

When performing a DNA Melting measurement, most of the samples are only available in extremely small amounts. Due to a limited amount of sample, it is essential that the amount used for measurement/analysis purposes be as little as possible. However, when sampling a small amount in a high temperature range, volatilization of the sample occurs, frequently complicating the analysis process.

Additionally, in order to increase the accuracy of the temperature readings of the samples, one of the 8-Position Micro Cell Block cells is utilized for the dedicated temperature monitoring so that the temperature in a cell can be used in the melting data.

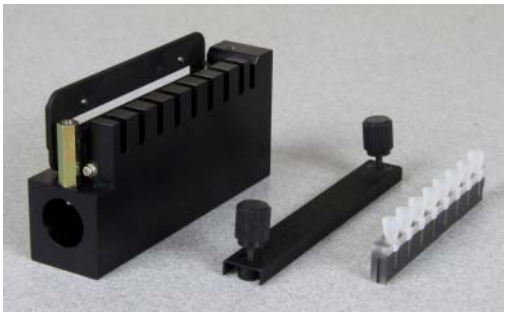

Figure 1. 8-position automatic cell changer (Left) / Lid retainer (Center) / cell and silicon lid (Right)

Measurement Systems
PAC-743 Water-cooled Peltier Cell Changer
1 mm 8-position micro cell block
1 mm 8-position micro cell
Silicon Cap (attached to 1 mm 8-position micro cell block)
Cap fixture (attached to 1 mm 8-position micro cell block)

Measurement program
VWTP-780 [Temperature Ramping Measurement /Melting Analysis] Program

Sample
Poly (dA-dT)
Poly (dA-dT) *pH7KH2PO4-NaOH* buffer solution (200 µg/mL)

Measurement Parameters

Start condition:	Keep within ±0.10 ºC of the temperature setting for 3 seconds
Data interval:	1 ºC (20 - 50 ºC), 0.1 ºC (50 - 70 ºC), 1 ºC (70 - 100 ºC)
Temperature gradient:	2 ºC / min
Response:	Fast
Wavelength for measurement:	260 nm

	Holder sensor:	Internal Cell Sensor:
Number of cells:	8	7
Temperature control sensor:	holder	holder
Temperature monitor sensor:	holder	cell (8)

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Results

The melting curves from the results of sample measurements with all eight micro cell using the holder sensor are plotted as shown in Figure 2. The time required for the measurement was totally 2.5 hours and the changes of samples volume during the measurement process are shown in Figure 3. During the measurement, Nujol was placed on top of the sample cells to prevent the sample from adhering to silicon caps.

After completion of the measurement of samples, the solution levels for each of the 8 cells were higher than the upper limit of cells (indicated using a red dotted line), and the decrease in sample volume were almost not observed by the human eye. In short, by using the silicon cap and cap fixture, volatilization of samples can be prevented.

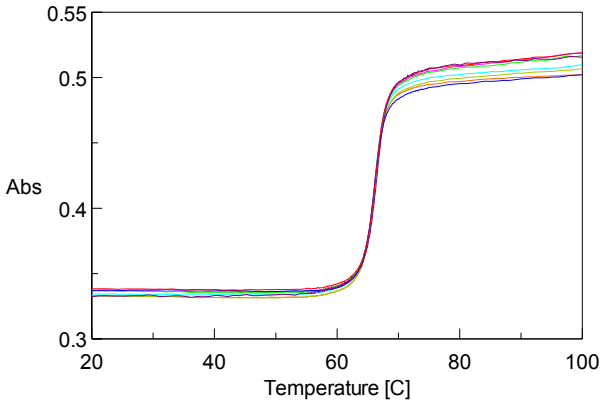

Figure 2. Melting curve data
[Plotted by using Holder Sensor]

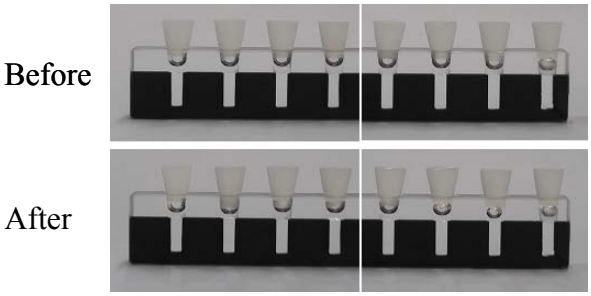

Figure 3. Change in sample volume before and after measurement
[Cell 1 from left]: Upper limit of cell

In order to enhance the accuracy of the temperature, one of the eight cells (referred to as “cell 8”) was used exclusively to monitor the sample temperature. Figure 4 shows a result of melting curves using the temperature readings from internal cell sensor. These temperature values were plotted in the horizontal axis in Figure 4 using data collected from internal cell sensor in cell 8. No evaporation was observed in this case.

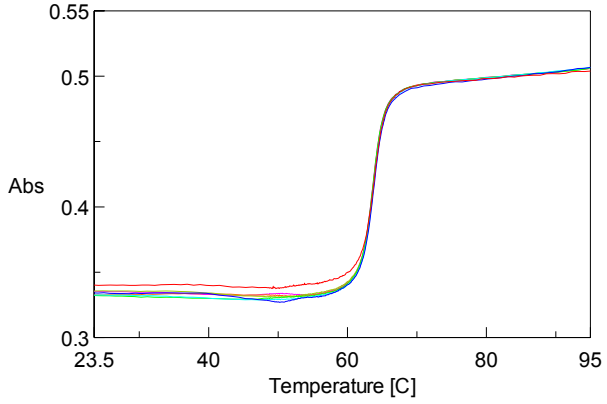

Figure 4. Melting curve data
[Plotted by using Internal Cell Sensor]

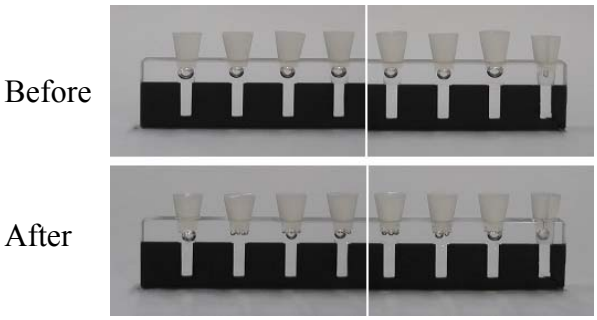

Figure 5. Change in sample volume before and after measurement
[Cell 1 from left]: Upper limit of cell

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The results of the melting points, calculated from the melting curves data in Figure 2 and 4, are shown in Table 1 and 2 below. The results using the holder sensor are shown in Table 1 ranging melting temperature between 66.0 ºC ~66.2 ºC (average 66.1 ºC) with standard deviation of 0.08 ºC and coefficient of variance of 0.13%. On the other hands, the results using internal cell sensor are shown in Table 2 ranging melting temperatures between 63.6 ºC ~ 63.8 ºC (average 63.7 ºC) with standard deviation of 0.08 ºC and coefficient of variance of 0.12%. This indicates that the temperature using the holder sensor was approximately 3.5 ºC higher than using the internal cell sensor. From these data, it can be concluded that the actual temperature of the holder was 3.5 ºC higher than the temperature of the original sample in the cell, while both the standard deviation and coefficient of variance had no major differences in their result.

In conclusion, to compare the melting temperatures relatively between each sample, the holder sensor is believed to be sufficient, while the internal cell sensor in the sample cell is ideal for measuring the absolute value of melting temperatures.

Table 1. Melting temperature
[Holder Sensor]

	Temp [ºC]
Cell 1	66.1
Cell 2	66.0
Cell 3	66.0
Cell 4	66.1
Cell 5	66.1
Cell 6	66.0
Cell 7	66.2
Cell 8	66.2
Ave.	66.1
S.D.	0.08
C.V.	0.13

Table 2. Melting temperature
[Internal cell Sensor]

	Temp [ºC]
Cell 1	63.6
Cell 2	63.6
Cell 3	63.6
Cell 4	63.6
Cell 5	63.7
Cell 6	63.7
Cell 7	63.8
Ave.	63.7
S.D.	0.08
C.V.	0.12

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