Application Note

UV-0016

DNA Melting Measurement with the PAC-743/743R Water-cooled Peltier Cell Changer Measurement with a Temperature Sensor in Cell

For the melting measurement of DNA samples, a temperature sensor can be inserted into the sample cells and the actual temperatures of samples plotted on the horizontal axis in order to increase the accuracy of the temperature readings for the melting experiment. This measurement technique is easy to be applied for 10-mm rectangular cells with a larger sample volume. However, for cells with a small sample volume such as the 8-position micro cell (100µl), a temperature sensor blocks the instrument optical path. It is then difficult to obtain both absorbance and temperature of a sample simultaneously.
Here, a DNA measurement example using the 8-position micro cell with a temperature sensor is outlined. By using one of the 8-position micro cells as a temperature monitor (Figure 1), the horizontal axis of the temperature course data can be plotted with actual temperatures obtained by the sensor. This increases the temperature accuracy of measurements with the 8-position micro cell.

Measurement System
PAC-743 water-cooled Peltier cell changer
8-position micro cell block
8-position micro cell
Silicon cap
Cap pressure fixture
Sensor in cell

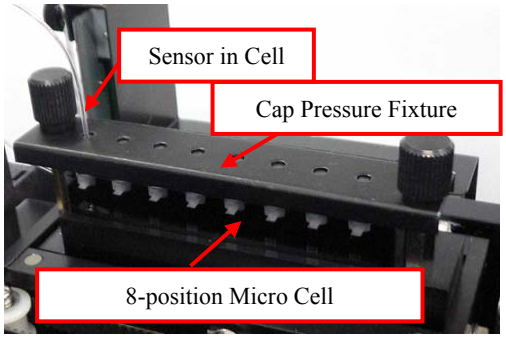

Figure 1: Sample Setting

Measurement Program
VWTP-780 Temperature Gradient Measurement Program

Sample
Poly (dA-dT)-Poly(dA-dT) pH7 KH_2PO_4-NaOH buffer solution (20 µg/mL)


Figure 2: Silicon Cap

Measurement Parameters
Number of cells: 7 *1)
Temperature control sensor: holder
Temperature monitor sensor: cell 8 *2)
Start condition: Keep within +/- 0.01-C of the target temperature for 3 seconds
Data interval: 1-C (20-50-C), 0.1-C (50-70-C), 1-C (70-100-C)
Ramp rate: 2-C/min
Response: Fast
Measurement wavelength: 260 nm
Reverse temperature measurement: ON

*1) Cell 8 was used only for temperature monitor. Absorbance was not measured.
*2) A silicon cap with a hole illustrated in Figure 2 was used for the temperature monitor cell. Silicon caps without holes were used for cells 1 to 7.

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Results

Measurement results are illustrated in Figure 3. The horizontal axis of the graph was plotted versus the values of the temperature monitor sensor in cell 8. The sample measurement required a total of 5 hours; 2.5 hours for the temperature increase data and 2.5 hours for the reverse temperature course. Table 1 indicates the melting temperature calculated from the temperature course data outlined in Figure 3. These results demonstrate melting temperatures for the various cells from 61.8 to 62.2ºC (Ave. of 6.20ºC), with a standard deviation of 0.13 ºC, and a coefficient of variance of 0.20%.

The same measurement was performed while obtaining sample temperatures using the standard temperature sensor (the holder sensor) within the PAC-743/743R accessory. Table 2 records the melting temperatures calculated from the temperature data using the holder sensor. Measurement results from this experiment provide a melting point varying from 63.0 to 63.3 °C that is around one degree higher than the temperature obtained when using the sensor in the sample cell. These results indicate that the temperature of the holder was around one degree higher than the actual temperature of the sample. On the other hand, the standard deviation and coefficient of variance are the same for both measurements. Considering these results, the holder sensor offers sufficient capability to measure a reproducible melting temperature for all sample cells. However, to obtain the absolute value for sample melts, a cell temperature sensor is highly recommended.

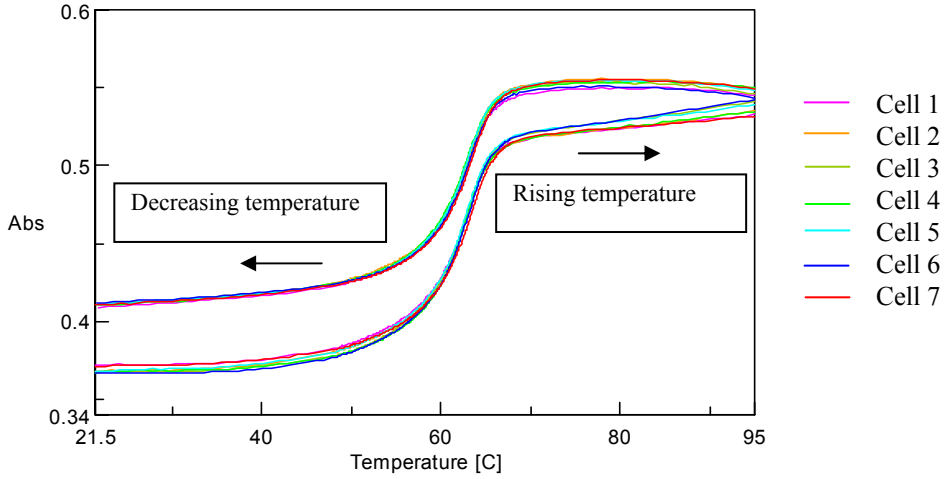

Figure 3: Temperature Course Data

Table 1: Melting Temperature (sensor in cell)

	Temperature (°C)	
	Rising	Falling
Cell 1	62.0	61.9
Cell 2	62.0	62.1
Cell 3	61.9	62.1
Cell 4	61.9	62.0
Cell 5	61.8	61.9
Cell 6	62.0	61.9
Cell 7	62.2	62.3
Ave.	62.0	62.0
Std. Dev.	0.13	0.13
C. V.	0.20	0.20

Table 2: Melting Temperature (holder sensor)

	Temperature (°C)
Cell 1	63.3
Cell 2	63.0
Cell 3	63.2
Cell 4	63.0
Cell 5	63.1
Cell 6	63.1
Cell 7	63.1
Cell 8	63.3
Ave.	63.1
Std. Dev.	0.13
C. V.	0.20

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