

DNA melting by One Drop measurement using capillary jacket

Melting measurement is one of remarkable measurement methods in Biotechnology field such as DNA melting and thermal denaturation of Proteins. For those measurements, 10mm rectangular type cell has been generally used, which requires large volume of sample for each measurement.

By using JASCO's water-cooled Peltier thermostatted cell holder PAC-743/743R with micro 8-position cell it is possible to measure sample with volume as small as 10 μ L *, however, for measurement of sample with much lower volume, it has been wished for a long time to have an accessory enabling such requirement.

*Refer to DNA melting measurement #3 in using of PAC-743/743R No.090911-012.

As one of solutions, JASCO introduces an application "DNA melting by One Drop measurement method" using JASCO V-630 BIO Spectrophotometer with capillary jacket for melting measurement, capillary and adaptor.

Capillary jacket for melting measurement

Capillary is disposal type quartz glass cell, whose optical pathlength is 0.5 mm and minimum sample volume is 3 μ L. After sampling by a capillary phenomenon, both edges of capillary are lapped by seal, which helps to avoid volatilization of sample. Such capillary is inserted into capillary jacket for melting measurement to be mounted to 6 channel cell block of Peltier cell holder or to PAC-743/ PAC 743R Water-cooled Peltier thermostatted cell holder. This capillary jacket has temperature sensor insertion port and temperature measurement in this port helps to measure accurate actual temperature of sample.

Measurement System

V-630 BIO	UV/VIS Spectrophotometer
ETCS-761	Water-cooled Peltier thermostatted cell holder with stirrer
OPS-515	Temperature sensor assy.
MCB-100	Mini water circulation bath
Capillary jacket for melting measurement, adaptor, capillary, seal material.	

Measurement Program

Temperature ramping / DNA melting program (Standard software of V-630 Bio)

Sample

Poly (dA-dT)-Poly(dA-dT) pH7 phosphoric acid buffer

Measurement Condition

Start setting:	3 seconds in the set temperature +/- 0.10 degrees C
Data acquisition interval:	0.1 degrees C
Temperature gradient:	1 degrees C / min
Response:	Fast
Measurement wavelength:	260 nm

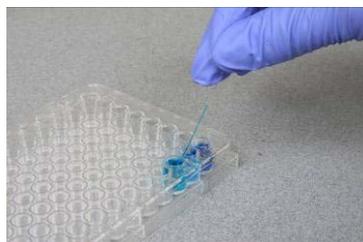


Fig.1 Capillary

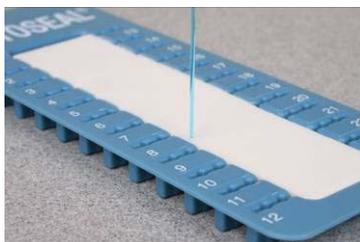


Fig.2 Capillary jacket (L) and Adaptor (R) for melting measurement.

Measurement Steps



Sampling by using capillary



Sealing both edges



Setting capillary jacket into cell holder



Setting temp. sensor and capillary into jacket



Capillary part

Result

Fig. 3 shows the result of measurement of Poly (dA-dT)-Poly(dA-dT).

The green spectrum is the measurement result data by using 10 mm rectangle type cell and blue one is the result data by using capillary cell. Temperature is measured by the temperature sensor inside of capillary jacket.

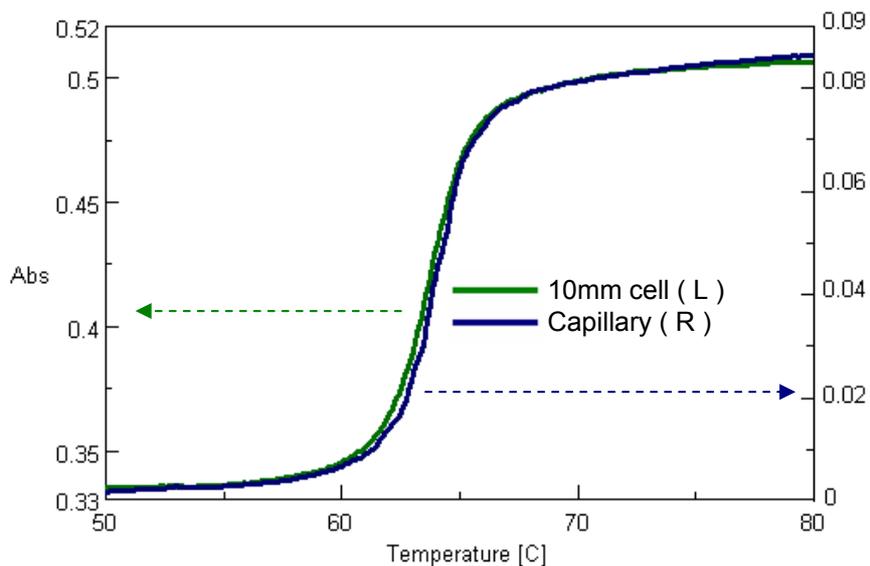


Fig. 3 Melting data

As the result of calculation based on above data for melting temperature, the similar results have been obtained for both measurement, such as 63.8 degrees C in 10 mm rectangular type cell and 63.9 degrees C in capillary cell respectively. It obviously proves that the measurement using small volume capillary is as reliable as measurement by general method using 10 mm cell.