

## CD Thermal Denaturation Measurement of Minute Sample Volumes

### Introduction

Circular Dichroism (CD) measurement is an effective tool for secondary structure analysis and the thermal denaturation analysis of proteins and nucleic acids. A rectangular cell of 1 mm optical path length is generally used for Far UV measurement, requiring approximately 200  $\mu\text{L}$  of sample volume. There has been great interest in an apparatus and technique to carry out CD measurements on very low volumes in the case of precious samples where only a tiny amount can be purified.

JASCO now offers a new capillary cell and capillary jacket for thermal ramping analysis of sample volumes less than 10  $\mu\text{L}$ . Measurement is simple and the capillary cells are inexpensive and disposable.

**Keywords:** microassay, capillary jacket, temperature controlled CD measurement, denaturation

### Sample preparation

1 mg/mL ribonuclease A aqueous solution is drawn into the capillary cell with a 0.5 mm optical pathlength and the capillary base is sealed. The cell is inserted in the capillary jacket for the CD measurement. A 0.5 mg/mL ribonuclease A solution using a rectangular cell of 1 mm optical path length is also measured for comparison.



Drawing sample\* into capillary



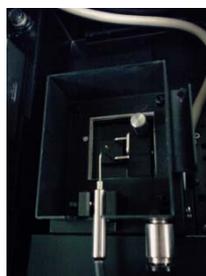
Sealing the sample



Ready for measurement



Insert the cell in capillary jacket



Set the capillary jacket in Peltier Thernostatted Single Cell Holder

\*The above pictures are using a colored sample for visibility, not the ribonuclease A solution.

## Measurement conditions

Measurement wavelength: 222 nm

Response: 8 second

Rising temp. rate: 1°C/min

Data sampling interval: 0.2°C

Spectral bandwidth: 1 nm

## Results

Fig.1 shows the thermal denaturation of ribonuclease A. Analysis using the JASCO JWTDA-519 Denatured Protein Analysis software calculates a denaturation temperature of 59.4°C for the capillary cell and is in accordance with 59.7°C for the rectangular cell. This result shows that the microassay for the capillary cell can be carried out with high accuracy.

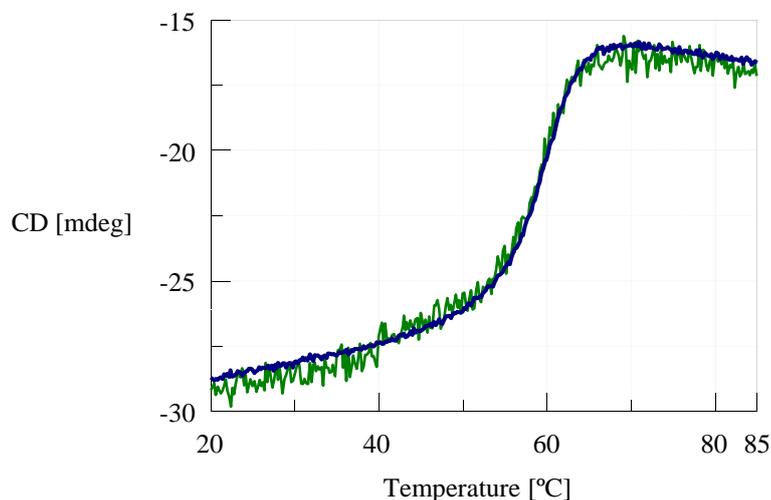


Fig. 1 Temperature control data of ribonuclease A

— capillary cell  
— 1 mm rectangular cell

## NOTE:

JASCO also offers the MSD-462 Micro Sampling Disk for spectral scanning measurements on sample volumes of 2 $\mu$ L to 10  $\mu$ L. The MSD-462 applications are shown in the following Application Notes: 260-CD-0011 and 260-CD-0019.