

RNA UV Absorbance Melts (Some Helpful Hints)

REFERENCE

JD Puglisi, I. Tinoco “*Absorbance Melting Curves of RNA*” (1989) Meth. Enzym. **180**, p.304.

BEFORE YOU MELT:

1. Wash cuvettes with 0.1 M NaOH and then with sterile ddH₂O so that the sides of the cuvette do not bead with H₂O but rather a sheen is observed along the walls when H₂O is added.
2. Autoclave buffers before use to completely degas the solution. This prevents gas bubbles from forming during the melt.

Note: Use buffers that do not have temperature dependent pKa profiles. **Tris** is a bad choice for a buffer, while **acetate** or **cacodylate** are good choices.

3. Make up the RNA sample so that initial OD₂₆₀ ~ 0.55 – 0.65 in the melting buffer.

THE MELT:

- 1.) Turn on the Cary300Bio, the temperature controller, and the N₂ tank.
- 2.) Open the “Cary WinUV” folder and select the “Thermal” icon.
- 3.) Go to “File” and select “Open Method.” Choose “Marino Melts” which is in the directory Varian/WinUV/
- 4.) Although the setup protocol will probably already be correct, one should check the following items under “Setup”:
 - a.) Cary file Wavelength should be 260nm.
 Temperature range should be set (i.e. 10-90°C).
 Rate = 0.25C/min.
 - b.) Accessories file Make sure “Cell 1” is selected. If melting many samples, select “Cell 2,” “Cell 3,” etc. as needed. Click “reset” to adjust the position of the cuvettes in the cells.
 - c.) Reports file – Name your sample.
- 5.) Put 1mL or more of ddH₂O in 2 quartz cuvettes and place them in cells 1 and 7. Press “Zero.”

- 6.) Place your sample(s) into the CARY300 block starting in position #1.
- 7.) Make sure “Probe 1” is inserted into one of the cuvettes with ddH₂O and placed in an unused well.
- 8.) Press “Start.”
- 9.) As soon as room temperature is reached, N₂ tank can be turned off.

AFTER YOU MELT (Processing Data):

- 1.) Under the “File” menu, select “Save Data As.” Save the file under the C:/ drive in the “People” folder (your own folder!) as a “*.csv” file.
- 2.) Exit the thermal window, turn off the temperature unit and the spectrometer, and disconnect the N₂ line.
- 3.) To open the file in Kaleidagraph, open the application and select “open.” Open the file and use the following settings: “Delimiter: Other (comma); Number: 1; Lines Skipped: 1.”