**Standard Operating Procedure: CD Sprectrometer**

**Date Updated:** May 10, 2024

**Updated By: JCM**

**Purpose:** Circular dichroism spectroscopy has been used for the determination of protein and nucleic acid secondary structures in solution. The machine is also capable of determining the temperature dependent changes in the protein or nucleic acid strucures.

**NOTE: The is not networked and has limited disk storage space. Only experiments of standard protein samples should be retained on the machine long-term. *Data from CMIC members will be exported to external drives for archiving.* Non-CMIC members will need to provide their own storage/backup scheme via the USB port. Regular maintenance may require the permanent removal of experimental data.**

Manufacturer: Jasco – J-1100 spectropolarimeter (Jasco, Easton, MD, USA):

Manual:

Consumables: None, but requires use of a cuvette available in the Kendrick or Raney laboratories

Signal readings:

**Initial Considerations:**

1. The CD spectrometer is located in the Kendrick laboratory with a secondary calendar and control through the Kendrick laboratory. Contact Dr. Kendrick or her designate for access.
2. For nucleic acid secondary structures, higher concentrations are required in higher volumes.

**Protocol: This SOP is a direct copy of the SOP developed by the Kendrick laboratory and the Byrd laboratory for G4 nucleic acids.**

**Circular Dichroism Spectra and Tm Analyses on Kendrick machine**

**Last modified:** 7/6/18 by AKB

CD Analyses on the Kendrick Lab/Dept. of Biochem. and Mol. Biol. Jasco – J-1100 spectropolarimeter (Jasco, Easton, MD, USA):

\*\*As of now, email Samantha Kendrick, at skendrick@uams.edu or Yingzhi Xu at yxu2@uams.edu to discuss availability of CD.

1. **PREPARATION OF SAMPLE**:
2. For G-quadruplex forming sequences:
3. Use 5 uM of DNA in a 200uL total volume of buffer
	1. E.g. if DNA stock is 100 uM: then 10uL DNA + 190uL of desired buffer
	2. Physiological KCl is ~140 mM
	3. For strong G4 formation a 10 mM Tris/100mM KCl pH 7.4-7.5 buffer is used
	4. CD buffer only: 10mM Tris pH 7.4-7.5

20-140mM KCl (different concentration to test G4 formation)

* 1. Kendrick CD/EMSA buffer:

10mM Tris pH 7.4

20-100mM KCl

2mM MgCl2

1mM EDTA

1mM DTT

0.1ug/ul BSA

0.1% Tween 20

10% glycerol

1. Briefly vortex and spin down
2. Heat to 95C for 5 min and slow cool (leave samples in heat block, remove block from heater and place on bench, and place new block in heater); allow to come to RT (takes ~3 hours)
3. Analyze on CD
4. **SPECTRA**
5. Sign in on CD log sheet posted on next to CD.
6. 30 mins before use, turn equipment on in this order:
	1. Temperature Cooling Unit (switch on right side)-black box behind instrument-small switch on top right
	2. CD itself (switch on left side)
	3. Computer – log on with your UAMS network ID and PSWD, open SPECTRA MANAGER program. Click on SPECTRUM MEASUREMENT (left hand menu). This will open a window that will turn the lamp on and initialize the CD. Skip the nitrogen purge. Allow lamp to warm up for **30 minutes**.
7. From the Measure Menu, select Parameters. On this screen there are 5 tab windows. On the first tab (General), you will need to change the settings for the CD. This means putting in the proper range for the wavelengths that are needed (220-320). Make sure that accumulation is set to 3-5, speed is 100nm/min. This is the proper scanning speed for publications.
8. LEAVE THE SECOND AND THIRD TABs (Cell unit and control) ALONE
	1. Under control check that baseline correction is not on and shutter open and close door automatically
9. The 4th tab (Info) is where you can input options. This can be viewed post run in case there are issues with the data later. You MUST input a concentration of your sample, but the blank is not necessary.
10. The 5th tab (Data) controls the saving of the file. You need to navigate to the folder you wish to save the file and name it.
11. Once the parameters have been set, click on the ok button and it will transfer the parameters to the machine.
12. Wash the cuvette with nuclease free, neutral pH water – 3X; acetone – 1X; water – 3X
	1. Dry the cuvette
	2. Rinse cuvette with buffer only – 2X
	3. Fill cuvette with buffer to measure a blank
	4. Wipe the outside of the cuvette with a Kim wipe, put in stopper, set it inside the chamber and close the lid. Click **blue “S” icon** to start run.
13. Once the blank has been run, remove the buffer, and add your first sample. There is no need to wash here, but wash with buffer or water/acetone in between each sample.
14. Measure Tm immediately after spectrum.

Data processing of spectra:

1. The spectral viewing program will automatically open when the run is complete. From this program:
	1. Drag sample spectra into sample spectra; confirm in top left hand-corner subtraction is correct; if not click Exchange Data; and click OK.
	2. Select the PROCESSING menu, select Correction, select Smoothing at desired maximum with the Savitzky-Golay method
	3. Select the PROCESSING menu, select CD options. Select OPTICAL CONSTANT. Click the box next to molar ellipticity, enter in optical length in cm and click OK.
	4. The new spectrum must be saved. Go to FILE menu, click SAVE AS, and name it. (recommend, same file name as original but with “\_Corrected” following)
2. To remove data off of computer:
	1. Select Export from file menu and save as a csv (or processing-common options-data dump-apply-copy)
3. **TEMPERATURE MELT CURVE**
4. Following spectra run; close SPECTRUM MEASUREMENT window and select VARIABLE TEMPERATURE MEASURMENT from left hand-side menu.
5. From the Measure menu, select Parameters
6. General tab:
	1. Select to perform the melt curve at the wavelength of the peak (the maximum molar ellipticity) from your oligo spectrum and enter the parameters below on the Temperature Tab:
		1. Start temp: 4C
		2. Target (C): 95C
		3. Interval: 1.0C
		4. Wait: 60s
		5. Gradient: 1C/min
	2. General
		1. Sensitivity: 100m deg standard – 200 mdeg/1OD is what I did – more sensitive might be better
		2. DIT: 8 sec
		3. Bandwidth: 1nm (default)
7. Ensure shutter for lamp is set to “auto” under control
8. Choose folder to save data and filename and start with Blue S.
9. To calculate Tm, need to export data as discussed above, then use GraphPad Prism to plot the data points and apply a nonlinear regression analysis, “log(inhibitor) vs. response – Variable slope (four parameters)”, this will calculate the midpoint at which 50% of the structure is melted (like the IC50). IC50 is provided in the results section of the Prism worksheet.

Turn off CD:

1. Close out of the SPECTRA MANAGER program
2. **Turn off CD first**
3. Turn off Temperature Cooling Unit
4. Log off computer