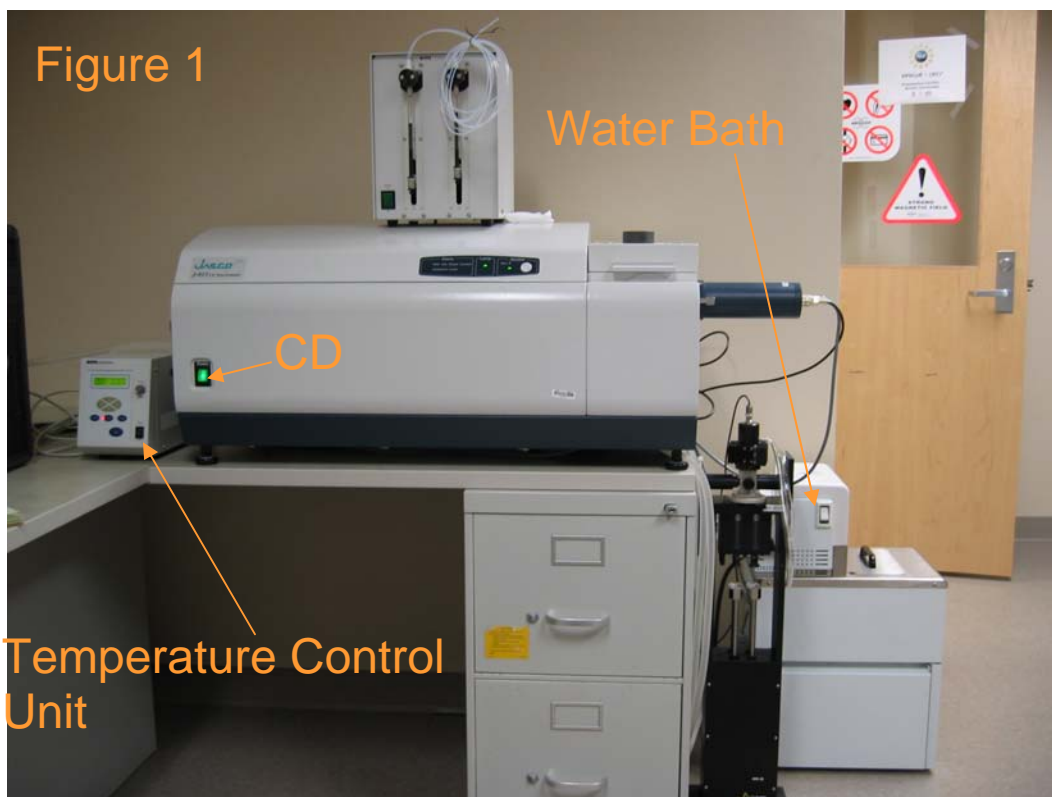


Protocol for Use of Circular Dichroism Spectropolarimeter (NSF/EPSCoR Proteomics Facility @ Brown University)

1. Getting Started

- Turn on water bath (Usually set at 20°C).
- Open the valve on the nitrogen tank so that the pressure gauge on the left side of the CD reads ~20 units.
- Wait for ~ 1 minutes to flush the instrument
- Turn on CD.
- Turn on Temperature Control Unit (Figure 1).
- Turn on computer/monitor.



2. Set Up – computer and temperature control

- Open the program *Spectrum Manager* on the desktop.
- To measure the spectrum, double click *spectrum management*.
- Instrument will be initialized and self tested. Lamp will be automatically turned on (will need ~5 min. for stabilization)
- Click the measurement tab at the top of the program and scroll down to **accessory**.
- Under accessories, click on temperature and scroll down to **JASCO Peltier Type** and chose this option.
- Click OK at the bottom.
- Click the Control tab at the top of the program and scroll down to accessory.

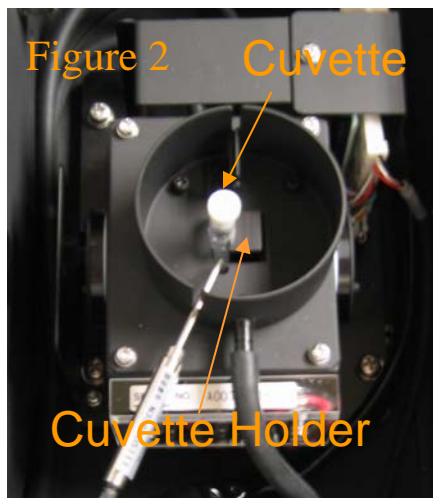
- Type 25.0°C (or your measurement temperature) into the temperature box, then click **apply**, then close at the bottom.

3. Setting Parameters

- Go back to the measurement tab and scroll down to parameters.
- Under the parameters tab set:
 - Sensitivity → Standard
 - Start → 260nm (This is a good place to start to study proteins, also, the starting point is always higher than the end)
 - End → 190 nm (One can get away with 195 nm because the end of the scan is not usually that informative; depending highly on salt concentration and used buffer; increase nitrogen flow if you want to measure below 190 nm; but usually not necessary to measure below 195 nm)
 - Data Pitch → 0.5 nm is standard but one can make the data pitch smaller for more accuracy (1 nm is good for a rapid, but informative spectrum)
 - Scanning Mode → Continuous
 - Scanning Speed → 50 nm/min is standard but one can make the speed slower for more accuracy or fast for a rapid check.
 - Response → 1 sec
 - Band Width → 1 nm
 - Accumulation → This is the number of scans you want the computer to do. If you chose more than one, the computer will run through the cycle that many times, then average the results.
- Under the data mode tab set:
 - Num. of Channels → 2
 - Channel #1 → CD
 - Channel #2 → HT
 - Additional channels can be added for Abs measurement and external pH.
- **IMPORTANT: Under the data file tab, chose a name and place you want to save the file. The data will be automatically saved to this place. If this is not correctly chosen it will override already recorded data!**
- Under the option tab, add any information or comments you want to be saved with your sample run.
- When done, click OK.

4. Loading Sample

- Total sample should equal approximately 600 µl at a concentration around 1-5 µM (slightly depending on your protein sample and your buffer etc.)
- When adding your sample to the cuvette, it is best to use a 100 µl pipetman. This is because the tip can get farther into the cuvette than a 1 ml pipetman, thus preventing any loss of sample due to temporary seals created at the neck of the cuvette.



- Once the sample is loaded, place the cap back on the cuvette and clean it with a kimwipe.
- Next, open the hinged lid on the right side of the top of the CD.
- In the middle of this space should be a black circular cap with two screws in the top. Remove this cap.
- Underneath this cap is a rectangular slot. On the right side of this slot, place the black rectangular cuvette holder with the circular hole in it. Make sure that the side with the hole is down when you place it in the slot and that light can pass through it

from left to right.

- Next, place the cuvette in this slot to the left of the cuvette holder (Figure 2).
- Replace the black cap, and the hinged lid.
- Leave this for a few minutes, then remove the cuvette and check for bubbles that have formed due to the temperature change. If any have formed tip the cuvette 45 degrees, and tap it carefully to dislodge the bubbles.
- Wipe the cuvette again and replace it.

5. Running a Scan

- Once the cuvette is replaced, the cap and lid are closed you can click the start button on the computer to start the run.
- The data will start to be graphed from right to left.
- The top graph is the CD, the bottom is the HT.
- A good CD graph will not dip below -50 (Unit)
- A good HT graph will not get above 800 (Unit) until around 200 nm or below
- If the CD goes below -50 (Unit) or the HT gets above 800 (Unit) before 200 nm, it may mean that the concentration of the sample is too high.
- These scans can be viewed in (program)

6. Shutting Down and Clean Up

- Remove cuvette and cuvette holder from CD, replacing the lid and cap afterwards.
- Dispose of sample by either recollecting it or throwing it out in the appropriate manner.
- Wash Cuvette with water, preferably using a vacuum cuvette washer.
- Turn off CD, Water Bath, Temperature Control Unit, and computer/monitor, but DO NOT turn off the gas
- Let gas run for 5 minutes to remove the ozone from the system, then shut off the gas
- You can save the data as a txt file (save as) and thus import in rapidly into MS Excel for further manipulation.

Advanced Measurements:

1. Variable Temperature Measurement

Variable temperature measurements are used to evaluate the melting point (stability) of a protein. A certain wavelength is monitored as the temperature changes.

- Perform “Getting Started” and “Set Up” exactly as outlined above.
- Go back to the measurement tab and scroll down to parameters.
- Under the parameters tab set:
 - Wavelength → 220 (This number depends a little on the protein being worked with, but this is a good standard)
 - Start → 25 degrees C
 - End → 90 degrees C (unless your protein is stable after 90 degrees, in which case, set this parameter higher).
 - Data Pitch → 5 degrees C is standard, but make this number smaller for more accuracy
 - Delay Time → 60 sec
 - Temp Slope → 5 degrees C/min is standard, make this smaller for more accuracy
 - Options → Check return to Start Temp
 - Sensitivity → Standard
 - Response → 1 sec
 - Bandwidth → 1 nm
- Under the data mode tab set:
 - Num. of Channels → 1
 - Channel #1 → CD
- Under the data file tab, chose a name and a place under which to save the run.
- Under the option tab, add any information or comments you want to be saved with your sample run.
- Load the sample according to “Loading the Sample” above.
- Click the start button on the program.
- When the run is finished, clean the machine the same as outlined in “Shutting Down and Clean Up” above.
- NOTE: One thing to note with these temperature measurements is that they can take a while depending on how the parameters are set and the computer program will stop recording data if it enters standby mode. Either set the computer not to go into standby mode, or stay active on the computer as to not lose your data.