PREPARING YOUR SAMPLE:

1. **Volume required**: for the 1 mm path-length cuvette, 400 ul is required to ensure beam coverage. For 2 mm path-length cuvette, 900 ul is required to ensure beam coverage.
2. **Cuvette**: Please ensure the cuvettes are clean. Protein aggregates frequently remain on the inner surface after simple rinsing, particularly after thermal melting experiments. Soak for 30 min-24 hours in the cleaning agent (2 % Hellmanex) and then rinse the cells thoroughly (5-10 times) with ddH20. Then final 2-3 rinses should be with 70% EtOH to ensure no smearing on the quartz surface. Dry the cuvette.
3. **Sample concentration**: It is always desirable to have an absorption level in the range 0.5-1.0 OD at the wavelength and path-length of interest. A ballpark starting concentration would be 0.5 mg/ml protein, or maybe half that for α-helical proteins and twice that for β-sheet proteins. If there is insufficient CD signal (< 5-10 mdeg), you should increase the concentration of the sample. However, since CD measures the difference between absorbance of orthogonally polarized light, using arbitrarily high concentrations will prevent enough light from passing through the sample for meaningful measurements. If the HT (dynode) voltage is > 600 V, the total absorbance of your sample is too high. It is possible to use even shorter path length (0.5-0.1 mm) cuvettes to solve this problem.
4. **Buffer consideration:** In the UV and particularly the far UV (< 250 nm), many buffer components absorb light, reducing the amount of light available for the measurement. If your buffer/solvent absorbs in the region of interest, it will add to the total absorbance (HT voltage) without improving signal. In particular it should be noted that chloride ions are strongly absorbing in the far UV and should be removed by buffer exchange (for example dialysis). Buffers must therefore be non-HCl based, and NaCl must be avoided if at all possible. Most proteins are stable in an equivalent concentration of fluoride, and high-purity NaF may be used up to a concentration of 150 mM, although < 50 mM will give superior results. 10 mM potassium phosphate is a good choice of buffer for most work. Low concentration of TRIS, sodium phosphate and borate are also reasonably transparent, but do not adjust PH with HCl. DTT, β-ME, or EDTA can be present at low concentrations (≤ 1 mM). SDS, CHAPS and octygluocoside (OG) are reasonably transparent detergents, but avoid Triton. No imidazole. If in doubt, run a UV wavelength scan on a spectrophotometer first; the OD should be < 1.0 over the nm range of interest. The table below lists the absorbance of many commonly-used salts and buffers.

Compound	pH	No Absorbance Above	Absorbance of a 10 Mm solution in a 1.0 mm cuvette			
			210 nm	200 nm	190 nm	180 nm
$NaClO_4$		170 nm	0	0	0	0
NaF, KF		170 nm	0	0	0	0
Boric Acid		180 nm	0	0	0	0
NaCl		205 nm	0	0.02	> 0.5	> 0.5
Na_2HPO_4		210 nm	0	0.05	0.3	> 0.5
NaH_2PO_4		195 nm	0	0	0.01	0.15
Na Acetate		220 nm	0.03	0.17	> 0.5	> 0.5
Glycine		220 nm	0.03	0.1	> 0.5	> 0.5
Diethylamine		240 nm	0.4	> 0.5	> 0.5	> 0.5

NaOH	pH 12	230 nm	> 0.5	> 2	> 2	> 2
Boric Acid, NaOH	pH 9.1	200 nm	0	0	0.09	0.3
Tricine	pH 8.5	230 nm	0.22	0.44	> 0.5	> 0.5
TRIS	pH 8.0	220 nm	0.02	0.13	0.24	> 0.5
HEPES	pH 7.5	230 nm	0.37	0.5	> 0.5	> 0.5
PIPES	pH 7.0	230 nm	0.2	0.49	0.29	> 0.5
MOPS	pH 7.0	230 nm	0.1	0.34	0.28	> 0.5
MES	pH 6.0	230 nm	0.07	0.29	0.29	> 0.5
Cacodylate	pH 6.0	210 nm	0.01	0.20	0.22	

5. **Additional information**: bring several ml of spare buffer for baseline scans, rinsing the cuvette between experiments and for dilutions.