Date: Feb. 3, 2006
Author: A DeGiovanni
Edited by: R. Kim

Materials/Reagents/Equipment	**Vendor**
UV spectrophotometer	Shimadzu BioSpec-1601 or equivalent
50 µl Quartz cuvette	Requires 60 µl of sample.
Buffer for blanking and dilution	
Protein solution to be measured	
Extinction coefficient for the protein	
Molecular weight of the protein	

Procedure

1. If the clarity of the protein solution is not known, centrifuge it in a microfuge for 5 minutes, 14000 rpm, prior to taking any readings.

2. Turn on the spectrophotometer and allow it to self calibrate and warm up for 5 minutes. The UV280 readings may be taken in the photometric, protein/DNA or any mode that provides a discrete 280 nm reading.

3. If using a 50 µl cuvette, you may want to dilute the sample by mixing 2 µl l of protein + 58 µl of buffer in an eppendorf (1:30 dilution). Centrifuge the diluted sample if foam was formed during mixing.

4. Make sure the cuvette to be used is clean and dry. Place enough buffer into the cuvette to completely fill the window (need **60** µl in a 50 µl cuvette). Do not allow any bubbles to stay in the light path. Place the cuvette into the spectrophotometer and auto zero, or blank, the instrument.

5. Remove the cuvette from the instrument and remove the buffer. Place protein solution into the cuvette again being sure to fill the window and prevent bubbles from being in the light path. Record the UV 280 reading. The absolute UV280 reading should be between 0.1 and 1.0 in order for the reading to be considered valid. If the value is outside of this range, the protein solution should be concentrated or diluted to achieve the required reading. Dilutions should take place outside of the cuvette and be well mixed prior to measurement. Absorbance readings are affected by the light path length. Light path length is measured in centimeters, one cm being standard. The value for a specific cuvette should be printed on the cuvette. A shorter path length will produce a smaller absorbance using the same protein concentration. As a result, a cuvette with a different path length can be used to obtain a reading within range.

6. Once an acceptable OD280 has been obtained, the cell can be removed from the instrument and cleaned for storage. The OD280 value is used to calculate the protein concentration. The calculation is:

protein conc. (mg/ml) = [OD280 x multiplication factor] x dilution (if any) x 1/path length.

The **Multiplication Factor (MF)** is unique for a given amino acid sequence and is defined as the molecular weight divided by the extinction coefficient at OD280.

Example: A protein of molecular weight 31163.8 with a 280 nm extinction coefficient of 17210 has an OD280 of 0.897 read with a dilution of 1:1 in a 1 cm cell.

Multiplication Factor = 31163.8 ÷ 17210 = 1.81

Protein concentration =

0.897 x 2 (dilution factor) x 1.81 (MF) x 1/1 (path length) = 5.825 mg/ml

7. **UV Scan 340 – 220 nm.**

A scan can provide information about the state of the protein.

A clean protein should show a symmetric curve with a maximum at 280 nm. An asymmetric curve indicates that something is bound to the protein.

A shoulder at 320 nm indicates aggregation and can affect the 280 reading.

Calculate the 280/260 ratio. It should be between 1.4- ~1.7 .