The Second Week of the Plant Hormone Lab (*revised instructions*)

10. Harvest the discs after 7 days of incubation. Label 5 test tubes with numbers 1-5, and transfer the 5 discs from each dish into the corresponding tube. Retrieve tube number 6 containing the 5 discs that were stored in the freezer.

11. Add 10 ml of 80% ethanol to the discs in each of the 6 tubes. Cap each tube with a marble.

12. Place the tubes in 75-78℃ water bath for 35 minutes to extract the chlorophyll from the leaf discs. The water bath must be heated electrically. The alcohol is flammable, so do not use a Bunsen burner and do not allow an open flame in the area. The bath should be set up in a fume hood, if one is available.

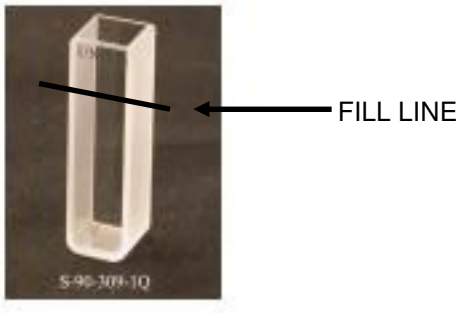
13. After 35 minutes, remove the tubes from the bath and allow them to cool.

14. Carefully, use forceps to remove the leaf discs (now white) from the tubes and discard them. Check the volume of each extract with a 10-ml graduated cylinder and add 80% ethanol to restore the volumes to 10 ml.

*Note: for the steps below, all solutions measured with a spectrophometer MUST be performed using the appropriate cuvette (a special 4-sided tube designed for spectrophometer use)

Spectrophotometer use:

A. AVOID touching a cuvette's clear sides (they are designed to have light pass through them)
B. Calibrate the spectrophotometer to A_{645} = 0.000 and A_{663} = 0.000 on blanks composed of 80% ethanol. To prepare a blank, take a clean cuvette and fill it approximately 4/5th full (see diagram below)



C. Align the front of the cuvette (usually marked with an arrow or fill-line) with the front of the sample compartment of the spectrophotometer.
D. Next, firmly seat the cuvette (but do not force it) in the sample compartment.
E. Remove the cuvette by holding the sides and gently pulling it straight up, out of the sample compartment

15. For each pigment extract, fill a clean cuvette 4/5th full, wipe the outside with a Kimwipe, measure and record in Table I the absorbances at 645 nm and 663 nm.

16. For each extract, calculate the combined concentrations of chlorophyll a and b according to the formula:

$$(\text{chl a + b})\ (\mu g/ml) = 20\ A_{645} + 8\ A_{663}$$

17. For each extract, calculate the final mass of chlorophyll a and b per mg of initial fresh mass. To do this, use the combined mass of the 5 discs, as measured the previous week, and the fact that the alcohol extracts 10-ml volumes. Thus:

$$\frac{(\text{chl a+b})\ \mu g}{\text{fresh mass mg}} = \frac{(\text{chl a+b})\ (\mu g/ml) \times (10ml)}{\text{mass of 5 discs (mg)}}$$

18. For extracts 1-5, calculate the final amount of chlorophyll per fresh mass as a percentage of the initial amount, i.e., divide (chl a + b) / fresh mass for each extract by the (chl a + b) / fresh mass of extract 6.