# Photosynthesis Pre-Lab Questions

1. In an intact leaf, relate the steps that usually occur once light is absorbed by the pigments.

2. DPIP is used to replace what molecule in the light reactions?

3. Explain why DPIP is being used as a substitute in this particular experiment.

4. Do you expect to see more or less transmittance of light if photosynthesis is actually occurring? Explain.

5. Do you think it's important how / where you hold the cuvettes and how you place them into the colorimeter? Explain.

6. Explain how you will calculate the rate of photosynthesis in this lab. (Be specific!)

7. Brainstorm at least 3 variables that might affect the rates of photosynthesis in this lab that you will be able to design an experiment for.

# Photosynthesis Lab Background Information

The process of photosynthesis involves the use of light energy to convert carbon dioxide and water into sugar, oxygen, and other organic compounds. This process is often summarized by the following summary reaction:

$$6\ H_2O + 6\ CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2$$

This process is an extremely complex one, occurring in two stages. The first stage, called the *light dependent reactions of photosynthesis*, requires light energy. The products of the light reactions are then used to produce glucose from carbon dioxide and water. Because the reactions in the second stage do not require the direct use of light energy, they are called the *light independent reactions of photosynthesis*.

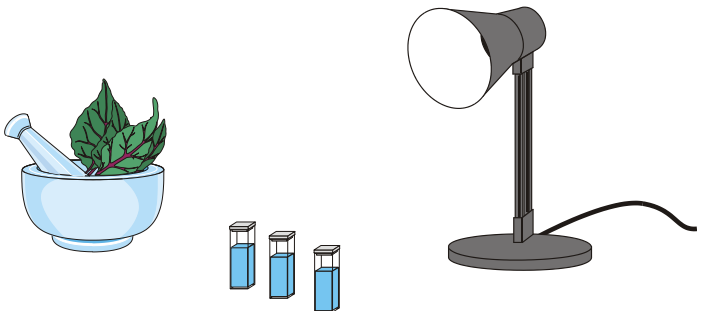
In the light reactions, electrons derived from water are "excited" (raised to higher energy levels) in membrane protein complexes called photosystems I and II. In both complexes, chlorophyll absorbs light energy that is used to excite the electrons. Normally, these electrons are passed to a cytochrome-containing electron transport chain. In the first photosystem, these electrons are used to generate ATP. In the second photosystem, excited electrons are used to reduce the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH). Both ATP and NADPH are then used in the light-independent reactions to produce glucose.

In this experiment, a blue dye (2,6-dichlorophenol-indophenol, or DPIP) will be used to replace NADPH in the light reactions. When the dye is oxidized, it is blue. When reduced, however, it turns colorless. Since DPIP replaces NADPH in the light reactions, it will turn from blue to colorless when reduced during photosynthesis.

## Objectives

In this experiment, you will:

- use a colorimeter to measure color changes due to photosynthesis.
- study the effect of different variables on photosynthesis.
- design an experiment to test factors affecting the light reaction for photosynthesis.



## Materials


LabQuest
Vernier Colorimeter (spectrophotometer)
cuvettes w/ lids (these go in colorimeter)
aluminum foil
DPIP/ phosphate buffer solution (use 2.5mL increments)
Chloroplast suspension (blended spinach mixture) (use 3 drop increments)
transfer pipettes
Anything else around the lab that you'd like to use (Ask about availability of additional materials)

## Set-up

1. Plug the colorimeter to one of the channels on the LabQuest. A meter window will display the absorbance readings from the colorimeter. Click the right arrow on the colorimeter so the 635nm display lights up.

2. You are now ready to calibrate the Colorimeter. Prepare a *blank* by filling a cuvette 3/4 full with distilled water. To correctly use a Colorimeter cuvette, remember:
   - All cuvettes should be wiped clean and dry on the outside with a tissue.
   - Handle cuvettes only by the top edge of the ribbed sides.
   - All solutions should be free of bubbles.
   - Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the Colorimeter.

3. Calibrate the Colorimeter.
   - Holding the cuvette by the upper edges, place it in the cuvette slot of the Colorimeter.
   - Press the AUTO Cal button. Wait until the display zeros.

**The rest of the methods will be designed by you and your lab group the day before the lab.

***Controlling for the heat emitted by the light source:
When setting up for photosynthesis reaction, you need a 600ml beaker or water to arrange between the energy source and your cuvettes as a heat sink if your source of energy is too warm for the choloroplast suspensions.

****Measuring using the colorimeter:
After placing the cuvette in the colorimter, allow 10 seconds for the readings displayed to stabilize. Record the absorbance value. You might have to repeat these steps after different time intervals to compare the rate of photosynthesis. Your negative control must be replaced in foil sleeves in between readings.

## - Photosynthesis Lab Design

After your discussion in class with your lab partners, you will decide on the procedure you are going to follow, obtain my approval, and then type up the procedure and bring it to class with you the day of the lab. You will submit the finalized procedure with your lab report the following week.

1. Discuss the variables to photosynthesis rate that each of you came up with in answering the pre-lab questions. Think about how you might actually test these variables and narrow your choices down to two possible variables that you might feasibly test during our next long block. Write these two variables down.

2. Share your ideas with me and I'll help you narrow it down to one. Write down the one variable that we have decided upon.

3. Write out the purpose/ objective of your group's experiment.

4. Write out your group's *testable* hypothesis. State the hypothesis such that the SPECIFIC predictions concerning your results are explicit. Use the "If…then" format for your hypothesis.

5. Describe at least 3 ways in which your experiment will be "controlled."

6. Make a list of materials that you will need for your experiment that are NOT already on the materials list provided with the lab.

7. Write out a detailed step-by-step protocol/ procedure to test your hypothesis.

8. Create a table to be used to collect your raw data. Remember that reproducibility is important. You will perform as many trials as time allows.)

# Photosynthesis Post-Lab Questions/ Analysis

These post lab questions should take you on the analytical process of revising your procedure as IF you had time to re-do your experiment. These would be your next steps if you wre doing this experiment as a "real" scientific investogator, rather than as a high school student constrained by a block schedul and school-year time frame. Remember that data can't be "wrong." It's just data. If it isn't what you expected, figure out why not. If it is what you expected, what does it mean and what would you do next?

1. Analyze your results: Are there trends in your data sets?
    a. If so, what are they (be SPECIFIC for each data set)?

2. How well do these trends in your data correlate with your hypothesis (again, be SPECIFIC)?
    a. Remember that you do not *prove* a hypothesis. Nobody does. Ever. Your data either supports, or fails to support, your hypothesis.

3. What did you use as your negative control?
    a. Were the results of the control data set consistent with your prediction?
    b. Explain the value of using a negative control in this lab. What light does this shed on your experimental design/ set-up?
    c. If didn't factor a negative control into your experimental design, explain what you would do for a negative control if had the chance to re-do the lab, describe the expected results of such a trial.

4. Explain your results: If your results seem to make conceptual sense, explain them in terms of these biochemical/ physical concepts.
    a. If your data does not make conceptual sense (or if some of your data sets do not), speculate on potential errors. Try to correlate specific unexpected data with specific procedural or design flaw. Under *no* circumstances should the phrase "human error" appear in your lab reflections and analysis!

5. Think about which parts of your design seemed to be successful and which parts did not.
    a. Using your typed procedure document, add in specific comments/ criticism about the different aspects of your procedure (you may either write these in by hand, or by adding typed comments to the document file).
    b. Revise this procedure document, in light of your analysis. Type in another color or font (or italics/ bold) your new steps or changes (to differentiate this from the original steps or parts that still remain).