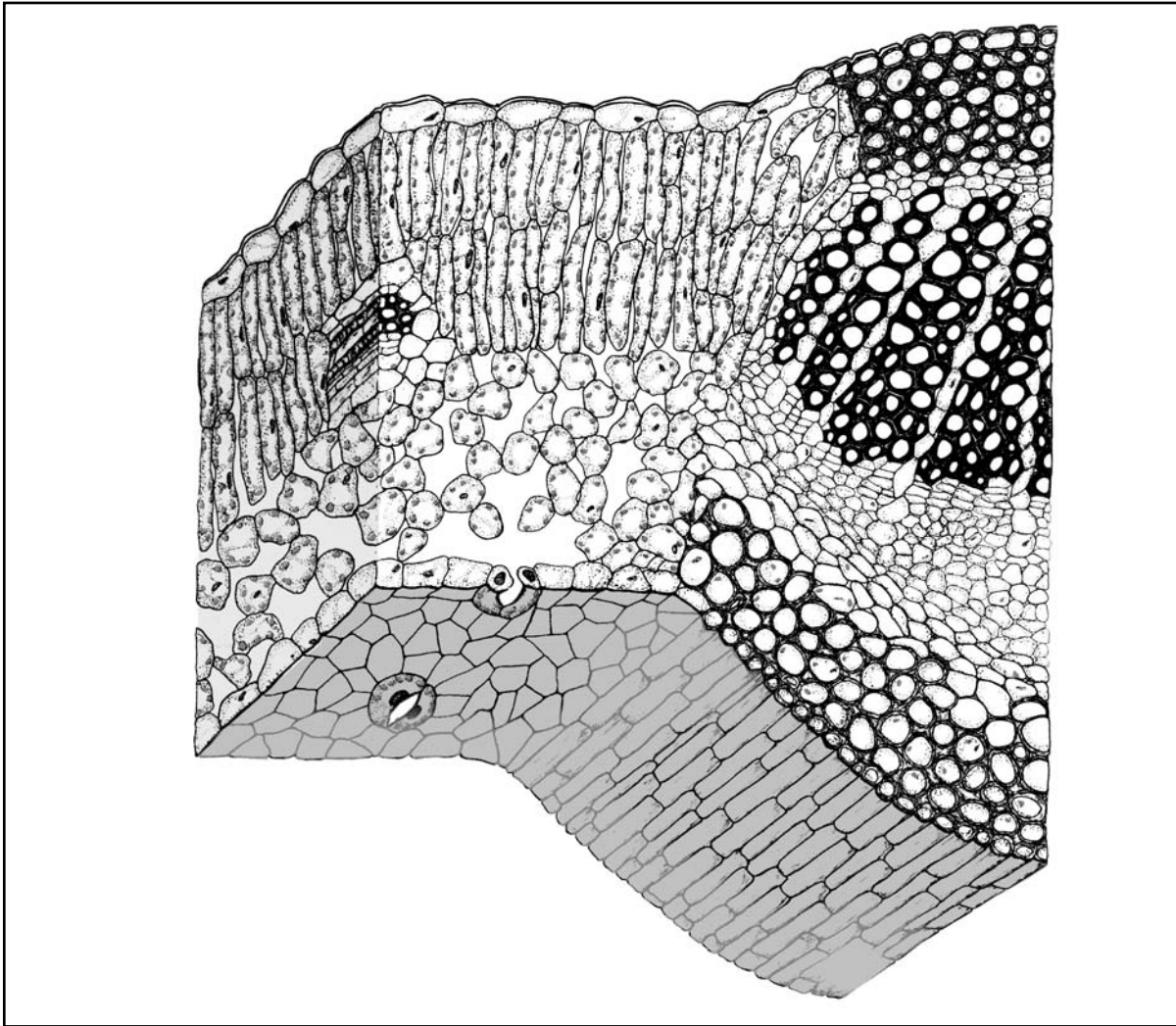


74-4676

# Leaf Disk Assay

TEACHER'S MANUAL



# Leaf Disk Assay

## Teacher's Manual

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# Leaf Disk Assay

## Overview

In this laboratory, students will

- study how plants convert sunlight into chemical energy while also releasing oxygen.
- measure the rate of photosynthesis using varying light intensities.

## Objectives

- Understand the principles behind the light-dependent reactions involved in photosynthesis
- Use a photosynthetic chamber to study the effect of baking soda (source of dissolved carbon dioxide) on the rate of photosynthesis
- Describe the need for light and carbon dioxide on the light-dependent reaction of photosynthesis

## Content Standards

This kit is appropriate for Advanced Placement® high school students and addresses the following National Science Content Standards:

### Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

### Life Science

- The cell
- Matter, energy, and organization in living systems

## Time Requirements

This laboratory requires one 45-minute period. This period does not include time required for the Preparation and Questions for Review sections.

## Safety

Use this kit only in accordance with prudent laboratory safety precautions, including approved safety goggles, lab aprons or coats, and gloves. Know and follow all school district guidelines for lab safety and for disposal of laboratory wastes.

## Materials

### *Included in the kit*

- 16 5-oz cups
- 1 bag of baking soda (1 tsp)
- 16 straws
- 16 10-mL syringes
- 16 syringe stands

### *Needed, but not supplied*

- plant leaves (spinach leaves recommended)
- 2 containers of water (at least 500 mL each)
- grow lights, lamps, or flashlights
- permanent marker or china marker

**Note:** This kit does not require the use of a spectrophotometer.

## Preparation

1. Photocopy the Student Guide for each student or pair of students.
2. Review the information in the Background and in the Procedure for Constructing and Using a Photosynthetic Chamber (see the Student Guide).
3. To prepare the baking soda solution, dissolve the entire bag of baking soda in one of the containers holding at least 500 mL of water. Do not dissolve baking soda in the other container of water.
4. Using the 5-oz cups provided, allocate 50 mL of the baking soda solution to the pairs designated to use it. Do the same with the water for the pairs designated to use it. Label the cups. The remaining solution and water may be used to repeat the procedure, if necessary.
5. If cut leaves are to be used, they should be refrigerated until you need them. Spinach leaves are recommended, but any tender, dark green leaf will work. Pairs will need enough leaves to cut out 10 disks.
6. Divide the class into two groups. Have students work in pairs within each group. Assign each pair of students one of the following treatments:
  1. Bright light/baking soda solution
  2. Room light/baking soda solution
  3. No light/baking soda solution
  4. Bright light/water
  5. Room light/water
  6. No light/water

**Note:** Because of class size, the class can be divided into two groups to make data comparisons more manageable. Treatments can be repeated if class size allows.

### Light Intensities

- Pairs using bright light may use a grow light, lamp, or flashlight.
- Pairs using room light will need only to place their chambers on their desks.
- Pairs using no light source must place their chambers in a dark area.

**Questions for  
Review  
Answer Key**

1. What does a plant need for photosynthesis?  
*Carbon dioxide, water, and light energy.*
2. a. What are the products of photosynthesis?  
*CH<sub>2</sub>O (sugars).*  
b. What is a by-product of photosynthesis?  
*Oxygen.*
3. Where does photosynthesis occur in a plant?  
*Photosynthesis occurs in the chloroplast organelles, which are found in the mesophyll cells of leaves.*
4. Write a hypothesis that this experiment is designed to test.  
*As light intensity increases, the rate of photosynthesis will increase.  
An increase in the level of dissolved carbon dioxide will increase the rate of photosynthesis.*
5. Which syringe serves as a control?  
*The syringe with water and no light source will serve as the control.*
6. What variables are tested in this experiment?  
*Light intensity and carbon dioxide levels.*
7. Compare the test groups. Which syringe had the most leaf disks floating after 20 minutes?  
*Results may vary. The syringe with a baking soda solution and bright light should have the most leaf disks floating after 20 minutes.*
8. Were there any syringes without floating disks?  
*Results may vary. The syringes without a light source should not have floating leaf disks.*
9. How do floating disks correspond to the rate of photosynthesis?  
*As photosynthesis occurs, oxygen is released. An accumulation of oxygen in the leaf disks will cause them to float. As photosynthetic rates increase, more oxygen will be released. The rate of photosynthesis should correlate with the intensity of the light provided.*
10. According to your data, does light intensity affect the rate of photosynthesis? Explain.  
*Results may vary. As photosynthetic rates increase, more oxygen will be released. The rate of photosynthesis should correlate with the intensity of light provided.*
11. How did the baking soda solution affect photosynthetic rates?  
*Results may vary. Baking soda will release carbon dioxide when dissolved in water. Carbon dioxide is needed for photosynthesis to occur; thus an increase in CO<sub>2</sub> may increase the rate of photosynthesis.*
12. Why is photosynthesis a light-dependent reaction?  
*Chlorophyll molecules absorb light energy and begin the process whereby carbon dioxide is fixed into more complex molecules. This reaction will occur only if light energy is available.*

## Extensions

- Test for the effect of different wavelengths of light on photosynthetic rates. Determine which colors are necessary for photosynthesis to occur by covering syringes with different color plastic film.
- Have students compare the photosynthetic equation with that of cellular respiration. Compare the reactants and products of each. Have students explain the need for both chemical reactions in plants.

## Resources

### Internet

Photosynthesis

[www.biology.clc.uc.edu/courses/bio104/photosyn.htm](http://www.biology.clc.uc.edu/courses/bio104/photosyn.htm)

Photosynthesis and Plant Cells

[www.educationalimages.com/it070007.htm](http://www.educationalimages.com/it070007.htm)

### Print

Rao, Krishna, and Hall, David O. *Photosynthesis*, 6th ed., Cambridge University Press, Cambridge, UK, 1999.

## Related Products

RN-30-3838 Privet Leaf, c.s. (slide)

RN-74-6470 Lab 4: Plant Pigments and Photosynthesis

RN-74-6474 Plant Pigments and Photosynthesis Instructor's Video

RN-74-0007 Lab 4: Plant Pigments and Photosynthesis,  
Carolina™ AP® Biology CD-ROM Series

## Leaf Disk Assay

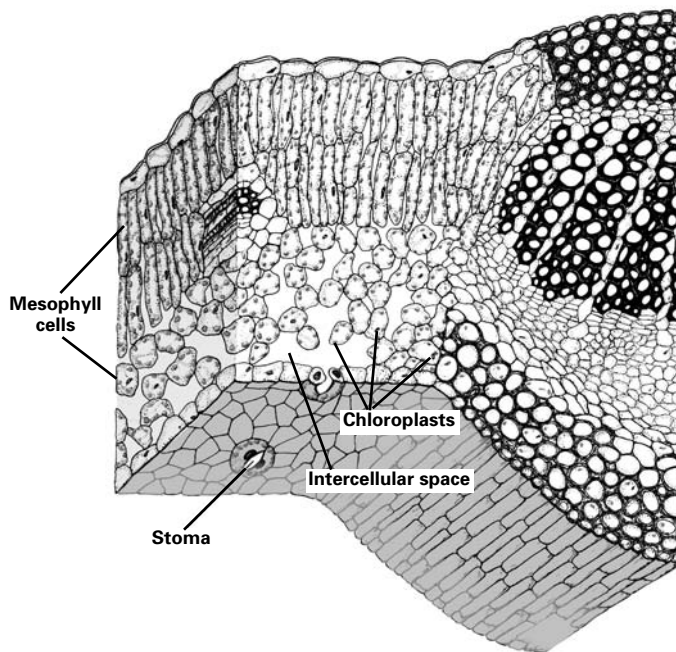
### Background

All living things need a source of outside energy. Animals get their energy from the food they eat. Plants obtain this energy from sunlight and convert it into sugars in the process called *photosynthesis*.

Plants capture sunlight using chlorophyll molecules found in the chloroplasts of their cells. Chlorophyll gives plants their green color. The highest concentration of chloroplasts is found in plant leaves. A leaf can be thought of as a solar antenna, an adaptation that allows the plant to collect as much sunlight as possible.

Chlorophyll molecules absorb the sun's energy and start the process whereby carbon dioxide is fixed into more complex molecules (sugars). The sugars produced are food not only for the plant producing them, but for other living things. Therefore, the energy that we obtain from our food ultimately comes from the sun.

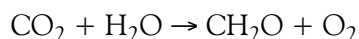
Within each leaf are cells containing chloroplasts. These cells, called mesophyll cells, are the plant's main sites for photosynthesis. A cross section of a plant leaf reveals tight layers of mesophyll cells, again an adaptation allowing plants to collect more sunlight. Below the tightly packed cells are more loosely arranged mesophyll cells that allow for intercellular spaces. These spaces are continuous, with small openings in the leaf surface called stomata (plural for "stoma"). Stomata allow gases to enter and exit the leaf.



Internal Structure of a Dicot Leaf

For photosynthesis to occur, carbon dioxide is also needed. This gas is found in the atmosphere and can enter the leaf through the stomata and then diffuse into the mesophyll cells.

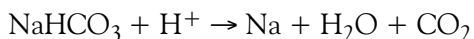
Using the energy from the sun, photosynthesis occurs by fixing carbon dioxide into organic molecules like glucose (sugar) shown in the following reaction.



As you can see from the equation, oxygen is also produced by photosynthesis. This is very important to us since we could not survive without this plant by-product.

It is possible to investigate photosynthesis by carrying out an experiment that demonstrates the production of oxygen. In this experiment, you will remove all oxygen and carbon dioxide from a plant leaf. When leaves are exposed to light, photosynthesis will occur and oxygen will be produced. We can study the rates of photosynthesis by increasing the amount of light for our leaves.

Since photosynthesis needs carbon dioxide as well, we can study the effect of carbon dioxide supplies on the rates of photosynthesis. A small amount of dissolved carbon dioxide is normally present in water. A baking soda solution, sodium bicarbonate, will increase the amount of carbon dioxide dissolved in water, making it available to our plant leaves.



## Materials

*For each pair of students*

- 50 mL of sodium bicarbonate solution or water
- 1 straw
- 1 10-mL syringe
- 1 syringe stand

*Needed, but not supplied*

- Plant leaves
- Grow light, lamp, or flashlight

## Procedure for Constructing and Using a Photosynthetic Chamber

Follow the steps below to construct a photosynthetic vacuum chamber composed of a 10-mL syringe containing leaf disks and water or a water-baking soda solution. You will use a straw to cut out 10 leaf disks, and may need more than one leaf to make all 10 disks.

1. Pull the plunger out of the syringe.
2. To cut out a disk, cover a fingertip on one hand with part of a leaf, then with the other hand, press the end of a straw against the leaf and your fingertip.
3. Place each leaf disk into the syringe. You may need to blow on the end of the straw to remove the disk.
4. After 10 leaf disks are in the syringe, use the straw to position them at the bottom. Replace the plunger.
5. Place the syringe tip into your group's designated liquid (either baking soda solution or water). Pull the plunger back to fill the syringe to the 5-mL mark. (It will probably work best to overfill, tap the bubbles out, then expel the extra until you reach 5 mL.)
6. Push the syringe tip firmly onto the syringe stand and pull the plunger back until you feel vacuum pressure. This vacuum will pull any air from the spaces within the tissue of the leaf disks. You should see air bubbles form on the disks as you create more negative pressure.
7. Gently shake the syringe or tap it on the side of your desk while maintaining the vacuum. This will remove the air bubbles from the disks.

8. Slowly release the plunger. When you turn the tip of the syringe upward, the leaf disks should start to sink. You may need to repeat this process several times to get all 10 disks to sink. After the disks have sunk, remove the stand and fill the syringe to the 10-mL mark.
9. If using bright light, place your syringe with its tip upward about 10 cm from a grow light, lamp, or flashlight.
10. If using room lighting, place your syringe with its tip upward on a desk.
11. If using no light, place your syringe with the tip upward away from direct light. You may need to cover your chamber to ensure that it is not exposed to light.
12. After 2 minutes, tap the sides of your syringe and count how many leaf disks are floating. Continue to observe and record the results every 2 minutes for a total of 20 minutes.
13. Record your results on Table 1.
14. After completing your experiment, compare your findings with other student pairs in your group and fill in Table 2 with this data.
15. Graph your group's results. Title the graph and supply the following information:
  - a. The independent variable on the x-axis.
  - b. The dependent variable on the y-axis.

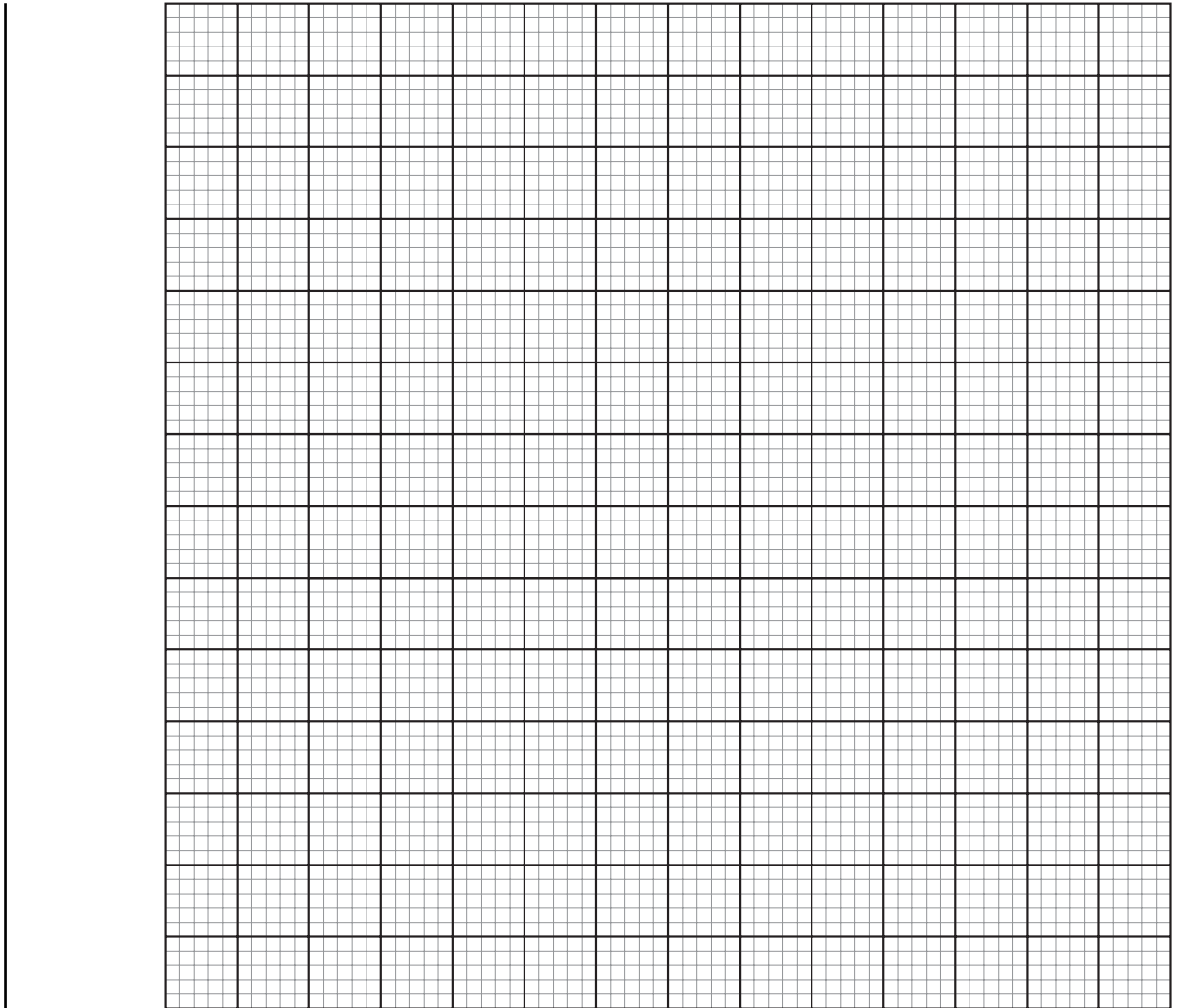
**Table 1: Individual Results**

<b>Treatment</b>	<b>Time in minutes</b>	<b>0</b>	<b>2</b>	<b>4</b>	<b>6</b>	<b>8</b>	<b>10</b>	<b>12</b>	<b>14</b>	<b>16</b>	<b>18</b>	<b>20</b>
	<b>Disks floating</b>											

**Table 2: Group Results**

	<b>Time in minutes</b>	<b>0</b>	<b>2</b>	<b>4</b>	<b>6</b>	<b>8</b>	<b>10</b>	<b>12</b>	<b>14</b>	<b>16</b>	<b>18</b>	<b>20</b>
<b>Treatment</b>	<b>Disks floating</b>											

Title: \_\_\_\_\_



## Questions for Review

1. What does a plant need for photosynthesis?
2. a. What are the products of photosynthesis?  
  
b. What is a by-product of photosynthesis?
3. Where does photosynthesis occur in a plant?
4. Write a hypothesis that this experiment is designed to test.
5. Which syringe serves as a control?
6. What variables are tested in this experiment?
7. Compare the test groups. Which syringe had the most leaf disks floating after 20 minutes?



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