

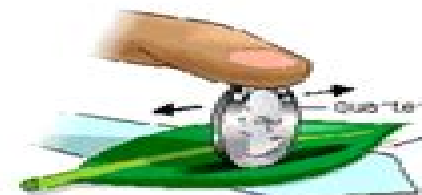
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AP Biology Photosynthesis Lab

Part A: Leaf Chromatography

Objectives

- Use chromatography to separate plant pigments
- Calculate R_f values from collected data



Background to Activity A

In science, it is often useful to know if a material is composed of a pure substance or if it is a mixture. Paper chromatography is a technique that is widely used to separate mixtures of biological compounds. In this activity, you will use paper chromatography to determine if a green leaf contains only one pigment or a mixture of pigments. You will do this by placing a line of leaf pigment near one edge of a sheet of chromatography paper. You will place this edge of the paper into a solvent (a mixture of acetone and petroleum ether). (**Caution:** Both acetone and petroleum ether are highly volatile and flammable. Keep the chromatography jar tightly capped except when inserting or removing the chromatography paper.) The solvent will be absorbed into the paper and encounter the line of pigment. If the pigment is a mixture of molecules, it is likely that some molecules will be more soluble in the acetone and others in the petroleum ether. Different molecules may attach more-or-less strongly to the cellulose of the paper through the formation of hydrogen bonds. If the leaf pigment is a mixture, you can expect that as the solvent travels up the chromatography paper, different molecules will dissolve in the solvent and travel up the paper at different rates. This will lead to the formation of different color bands on the paper, one band for each substance in the mixture.

Four pigments are widely found in leaves: carotene, xanthophyll, chlorophyll *a*, and chlorophyll *b*. Carotene is highly soluble in the solvent you are using. Its molecules do not form hydrogen bonds with cellulose. Carotene produces a faint yellow to yellow-orange band. Xanthophyll is slightly less soluble than carotene in the solvent. It forms some hydrogen bonds with cellulose. Xanthophyll produces a yellow band. Both chlorophyll *a* and chlorophyll *b* readily form hydrogen bonds with cellulose. Chlorophyll *a* gives a bright green to blue-green band. Chlorophyll *b* produces a yellow-green to dark, olive green band.



Figure 1

Part A Pre-Lab Questions - Read through the lab and then answer the following questions.

1. Explain how chromatography works.

Chromatography works when molecules of a mixture are rubbed onto a surface in order to separate the components making up the mixture.

2. What is the role of the pigments in leaves?

Pigments serve to help aid cellular processes in plants. Different pigments absorb different wavelengths to produce different colors. Leaf pigments also act as a large part in photosynthesis as they can help absorb light energy.

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1. Understanding the eBook Ap Biology Photosynthesis Lab Answers
 - The Rise of Digital Reading Ap Biology Photosynthesis Lab Answers
 - Advantages of eBooks Over Traditional Books
2. Identifying Ap Biology Photosynthesis Lab Answers
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Ap Biology Photosynthesis Lab Answers
 - User-Friendly Interface
4. Exploring eBook Recommendations from Ap Biology Photosynthesis Lab Answers
 - Personalized Recommendations
 - Ap Biology Photosynthesis Lab Answers User Reviews and Ratings
 - Ap Biology Photosynthesis Lab Answers and Bestseller Lists
5. Accessing Ap Biology Photosynthesis Lab Answers Free and Paid eBooks
 - Ap Biology Photosynthesis Lab Answers Public Domain eBooks

- Ap Biology Photosynthesis Lab Answers eBook Subscription Services
- Ap Biology Photosynthesis Lab Answers Budget-Friendly Options
- 6. Navigating Ap Biology Photosynthesis Lab Answers eBook Formats
 - ePub, PDF, MOBI, and More
 - Ap Biology Photosynthesis Lab Answers Compatibility with Devices
 - Ap Biology Photosynthesis Lab Answers Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Ap Biology Photosynthesis Lab Answers
 - Highlighting and Note-Taking Ap Biology Photosynthesis Lab Answers
 - Interactive Elements Ap Biology Photosynthesis Lab Answers
- 8. Staying Engaged with Ap Biology Photosynthesis Lab Answers
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Ap Biology Photosynthesis Lab Answers
- 9. Balancing eBooks and Physical Books Ap Biology Photosynthesis Lab Answers
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Ap Biology Photosynthesis Lab Answers
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Ap Biology Photosynthesis Lab Answers
 - Setting Reading Goals Ap Biology Photosynthesis Lab Answers
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Ap Biology Photosynthesis Lab Answers
 - Fact-Checking eBook Content of Ap Biology Photosynthesis Lab Answers
 - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks

14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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