

## Honors Biology: Chromatography Lab Answer Key

### Terms and Definitions (1 Point Each):

1. Chromatogram
2. Mobile Phase
3. Stationary Phase
4. Solute
5. Solvent
6. Heterogeneous Mixture
7. Homogenous Mixture
  
8. Endergonic Reaction
9. Exergonic Reaction
10. Heterotroph
11. Autotroph
12. Photosynthesis
13. Pigment
14. Adenosine Tri-phosphate
15. Absorption Spectrum
16. Cellular Respiration
17. Anaerobic Respiration
18. Aerobic Respiration
19. Fermentation
20. Lactic Acid Fermentation
21. Alcohol Fermentation

### Analysis Questions (2 Points Each):

1. In procedure A (ink portion), what was the mobile phase and what was the stationary phase?

The target molecule is placed on chromatography paper is the stationary phase. The mobile phase is the solvent, which in this case was denatured alcohol. The solvent rises up the paper, in other words the mobile phase travels along the stationary phase. When it reaches the sample, the solvent carries the sample with it and different components of the sample travel at different rates thus separating the sample into strips.

2. If you had an ink sample that did not separate into color bands during the procedure, what could be the reason for this?

An ink sample that does not separate into bands of varying colors may be composed of a single pigment or perhaps the target molecule was submerged in the solvent thus preventing the solvent from separating out the sample's pigments.

3. Why do some colors rise farther on the filter paper than others?

Several factors play a role in determining the distance that the colors rise depends on the size of the pigment molecule; which pigments are more soluble in the denatured alcohol; and the level of attraction that the pigment has to the chromatography paper.

4. What could it mean if pigments of the same color in ink samples from different pens have the same Rf values?

If pigments of the same color in ink samples from two different pens have the same Rf values, it is possible that both types of pen ink contain the same pigment molecule.

5. In procedure B (plant portion), which pigment migrated the farthest? Why?

This should have been carotene (yellow) because it is the most soluble in the denatured alcohol and it is the smallest pigment molecule. (If this was not the case for your lab group and another pigment migrated the farthest, please apply a logical explanation from question number 3.)

6. During the summer, leaves are generally bright green. What would you hypothesize that this indicates about the role of **green wavelengths**, **chlorophyll**, and the **photosynthetic process**?

Leaves display a bright green color because this is the wavelength of the light that chlorophyll is reflecting. Since green light is reflected and not absorbed, it indicated that this is the least useful wavelength in the visible light spectrum for the process of photosynthesis.

7. Design an experiment to test your hypothesis from the question above. Describe your experiment or draw a picture for your experiment setup. If you draw your setup, be sure to label each component and purpose.

Your experiment needs to include an independent and dependent variable. Must contain both experimental and a control group. Need to have a clear hypothesis/ purpose. Something that would support the theory that green wavelengths are the least useful in the process of photosynthesis.

8. Why do leaves change color in autumn?

During the autumn chlorophyll begins to break down, allowing the carotenes and xanthophylls to show their red, orange, and yellow hues.

9. What is the function of the chlorophylls in photosynthesis?

Chlorophylls a and b absorb blue and red light and reflect green. Chlorophyll a is the main photosynthetic pigment involved in the conversion of light energy to chemical energy.

10. What are the accessory pigments and what are their functions?

The accessory pigments are carotenes and chlorophyll b, which absorb light in a region of the spectrum different from chlorophyll a and transfer the energy to chlorophyll a. They work to “broad the spectrum of light” that chlorophyll a can absorb.

11. In your experiment, you used paper chromatography to separate various pigment molecules. There are several other chromatographic techniques employed to separate a variety of molecules. **Research another form of chromatography and describe it in depth.**

Need to research a method of chromatography other than paper chromatography. Looking for three to four sentences explaining the type of chromatography that you researched. (Topics: column, thin-layer, gas, high-performance liquid, ion exchange, gel filtration, or affinity chromatography.)

12. What does the Rf value represent? If you were to perform your experiment on a chromatography strip that was twice the length of the one you used, would your Rf values still be the same? Why?

The Rf value represents the ratio of the distance a pigment moved on the chromatogram relative to the distance the solvent front moved. Since it is a ratio, Rf values would remain the same on any size paper. Though the pigment may move a farther distance, the solvent front would as well.

13. Below is a list of five molecules and their various Rf values. Assuming the solvent front travelled 54mm, **draw and place each molecule where it would be found on a finished chromatogram.**

Must draw a chromatogram and place each molecule where it would be found based on your calculations.

Molecule	Rf Value	Distance Substance Travelled
N,N-fictionol	.41	22 mm
Cis-2,4-pretendium	.20	11 mm
dl-made-upelene	.72	39 mm
D(+)-tetra-imaginase	.91	49 mm
Polysynthetic acid	.78	42 mm