EDVOTEK®

THE **BIOTECHNOLOGY**
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Edvo-Kit #
1100

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Scents and Sense-ability

Experiment Objective:

The objective of this experiment is for students to understand that olfactory receptors respond to smells and transmit them as signals to the brain. Students will also be able to understand the principles of thin layer chromatography and how they apply to separation of olfactory compounds.

See page 3 for storage instructions.

IMPORTANT:
Components A - E are somewhat volatile (prone to evaporation) and thus are prepared upon request and shipped **up to one week before** the planned experiment. Contact customer service to request these components.

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Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

Components		Storage	Check ✓
A	Limonene	Room Temp.	❑
B	Anethole	Room Temp.	❑
C	Eugenol	Room Temp.	❑
D	S-Citronellal	Room Temp.	❑
E	Unknown	Room Temp.	❑

This experiment contains enough reagents for 10 lab groups.

IMPORTANT:
Components A - E are somewhat volatile (prone to evaporation) and thus are prepared upon request and shipped **up to one week before** the planned experiment. Contact customer service to request these components.

Reagents and Supplies

• Anhydrous Sodium Carbonate	Room Temp.	❑
• Potassium Permanganate	Room Temp.	❑
• TLC Paper		❑
• Transfer pipets		❑
• Microcentrifuge tubes		❑

Experiment Requirements *(NOT included with this experiment)*

- Pencils
- 100 mL beakers
- 400 mL beakers
- 15 mL conical tubes
- Gloves
- Rulers
- Saran wrap
- 100% Ethanol (200 or 190 proof)
- Distilled water

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

None of the experiment components are derived from human sources.

Background Information

SENSES AND THE HUMAN NERVOUS SYSTEM

A wide array of senses allow humans to perceive and interact with the surrounding environment. We interact with our environment using the five senses: sight, scent, touch, taste, and sound. Our senses are controlled by our nervous system, and we process sensory information in our brain. Our central nervous system is composed of the brain and spinal cord, and the peripheral nervous system is composed of nerves that run throughout our body (Figure 1, green). These peripheral nerves take in information from our environment and send that information to the brain. If you place your hand on a hot plate, for example, you immediately remove it because it feels dangerously hot. In fact, you remove it so quickly that you don't even have to think about it. How does your hand move so fast on its own? Neurons communicate to each other extremely quickly. The neurons in your hand which are programmed to sense heat send signals at a rate of 45 miles per hour! Your brain then quickly processes that information and sends a signal back down to your arm causing it to move away from the hot plate.

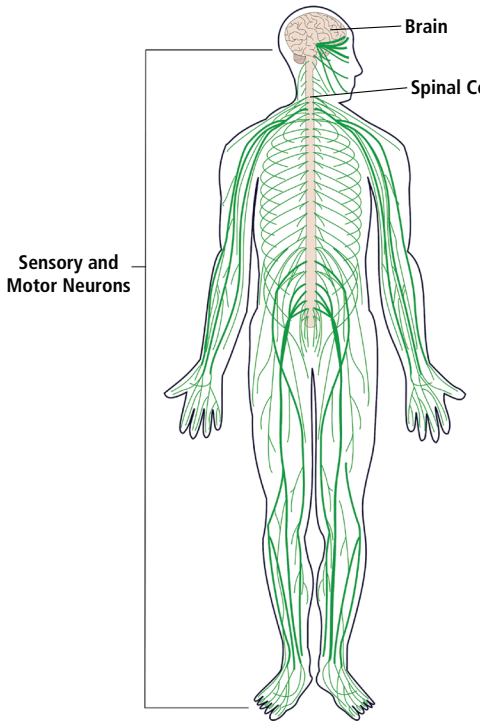


Figure 1: The nervous system

Our brain processes most of the information coming in from our environment in an area known as the thalamus. The thalamus is like the post-office for the brain, taking in information and sending it to the part of the brain where it belongs. Interestingly, the only sense that doesn't get processed in the thalamus is your sense of smell. Your sense of smell, or the olfactory system, brings information from your nose directly into your temporal lobe. The temporal lobe is responsible for processing sensory information and memories. As such, a certain smell can elicit feelings, memories, or recognition. This is a complicated system, and the 2004 Nobel Prize in Physiology or Medicine was awarded to Drs. Richard Axel and Linda B. Buck for their "discoveries of odorant receptors and the organization of the olfactory system".

Humans detect smells (odorants) through specialized sensory neurons that reside at the back of the nasal cavity (Figure 2). Odorants are actually small molecules that bind to sensory neurons in your nose. These sensory neurons contain odorant receptor proteins, and the amino acid sequence of the receptor protein creates a pocket to which an odorant molecule can bind. When activated by an odorant, the receptor protein activates a molecular switch, which then activates a series of cell signaling events. These downstream signaling events stimulate the neurons to fire, and a molecule's odor information is sent to the brain to be perceived.

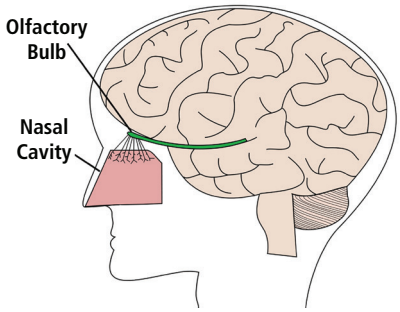


Figure 2: The olfactory system

Each binding pocket is specific for a given odorant, allowing similar molecules to have completely different smell profiles. As such, some similar molecules produce completely different scents because their

unique shapes stimulate different receptors. Scientists are able to describe smells using olfaction thresholds and visualize odorants using thin layer chromatography.

OLFACTION THRESHOLDS

Odors are classified using several different parameters. Four common parameters are concentration, intensity, offensiveness, and character.

- The **concentration** measures how much of an odorant must be present to be detected and identified as a particular smell.
- The **intensity** of an odor is the strength of the odor sensation.
- The **offensiveness** measures how favorable or unpleasant the odor is when smelled.
- The **character** of an odor is a description of how it smells – for example, a piece of bubble gum might smell minty, or perfume might smell floral.

These parameters influence one's experience with a given odorant.

While offensiveness and character of an odor may be subjective, scientists can quantitatively measure the concentration of odors. One way to do this is using gas chromatography mass spectrometry to determine which, and how much, volatile substances are present in a sample. However, a much simpler, and extremely sensitive, way to detect odors is to simply use one's nose!

There are two major ways that we can measure the concentration of odors with our nose: odor threshold number and recognition threshold. The odor threshold number is the amount of substance required for a scent to be detected. Alternatively, the recognition threshold is defined as the concentration where an odorant is recognized by 50% of humans. These can be found by diluting odors and testing a group of people to see at what concentration the odor can be recognized.

THIN LAYER CHROMATOGRAPHY

Although the nose is extremely efficient at detecting individual odors, identifying the components of a complex odor can still be difficult. Scientists can use other methods, such as Thin Layer Chromatography (TLC), to visualize the different components in a mixture of odorants. TLC is an analytical method used in chemistry and biochemistry for the separation and analysis of a wide variety of molecular mixtures. TLC methods can be used to separate mixtures of inorganic ions, organic molecules and bioorganic compounds such as pigments, lipids, amino acids, nucleotides and sugars. In this way, TLC methods can be used to separate odorants.

The TLC plate typically consists of a 0.1 mm thick layer of adsorbent material bonded to a glass or plastic support. The adsorbent consists of a microscopic layer of material. This surface provides a large area for chromatographic separation. After a small volume of sample solution is applied to the adsorbent surface and allowed to dry, the plate is placed in a beaker or tank containing the appropriate solvent. Only the edge of the plate nearest the samples is in contact with the solvent. The solvent is drawn into the dry adsorbent material and travels up the plate through the samples. The migration rate of the sample components over the adsorbent depends on their chemical structure.

Adsorption chromatography was discovered by the botanist Dr. Tswett in 1903. Common adsorption chromatography materials include magnesium carbonate, magnesium silicate, alumina and activated charcoal. Most adsorption materials can have surface charges and can absorb polar or polarizable molecules.

One example of a polar molecule is water. As shown in Figure 3, the molecule has no net charge. However, each individual atoms has a partial charge. The oxygen atom has slightly more negative charge than the hydrogen atoms, which consequently have slightly more positive charges. This is

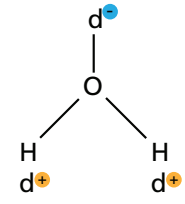


Figure 3: A water molecule

because the oxygen nucleus attracts the negatively charged electrons in the chemical bonds more strongly than the hydrogen nuclei. Therefore, even though the water molecule is overall electrically neutral, its individual atoms do possess partial negative or positive charges. Molecules that exhibit these properties are called polar. Molecules that contain opposite charges, or partial opposite charges, possess a dipole charge. The adsorbent material on the TLC paper has many polar and fully charged (ionic) chemical groups on its surface. Polar sample molecules can interact with these groups by dipole-dipole and dipole-ion interactions. These interactions involve the attraction between regions with opposite charges. Such interactions are shown in Figure 4.

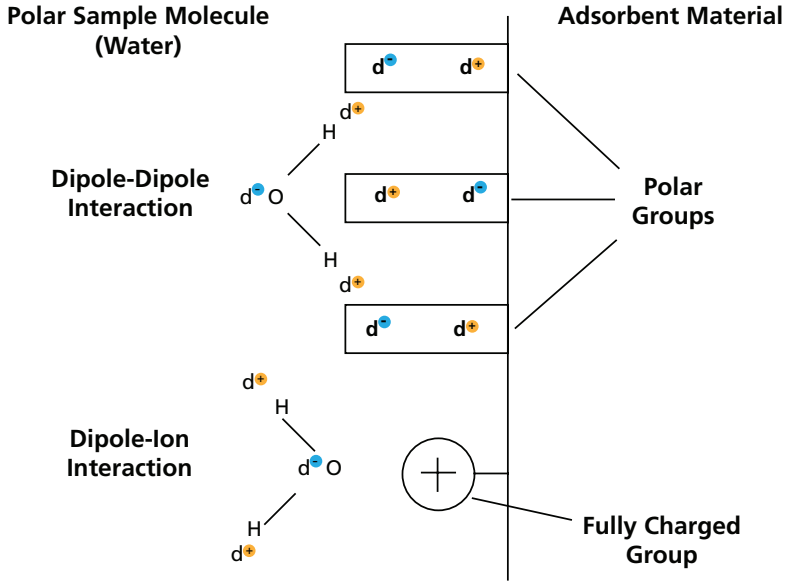


Figure 4

During TLC, molecules are separated by their attraction to either the adsorbant material or the solvent. The adsorbant material is referred to as the stationary phase, and the solvent is referred to as the mobile phase. Samples that have little or no solubility in the mobile phase and high solubility in the stationary phase will migrate slowly. Conversely, samples that have little solubility in the stationary phase but are highly soluble in the mobile phase will have the fastest migration rates.

Odorant molecules (examples in Figure 5) contain different types and amounts of charged and polar chemical groups. They also differ from one another with respect to their molecular weights, geometry, and the positions and numbers of carbon-carbon double bonds. This means that each odorant molecule will have a unique affinity for the stationary vs. mobile phase. The distance traveled by a sample from its origin divided by the distance that the solvent traveled is defined as the "retention factor (Rf)." A substance that does not migrate from the sample origin has an Rf = 0, while one that migrates completely with the solvent has a Rf = 1. Each odorant has a unique Rf, and the Rf of a compound can be used to identify it. Therefore, if there is more than one compound that makes up a particular odorant, you can separate them by TLC and determine their identity by the Rf value.

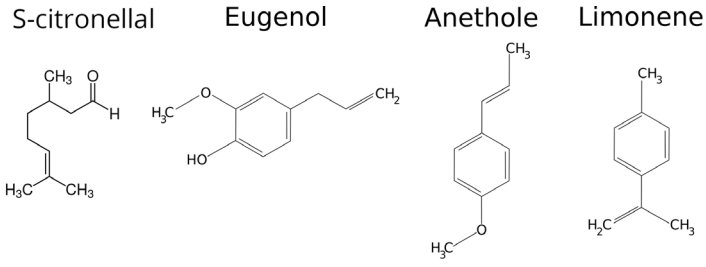


Figure 5

In this experiment, odorants will be identified, classified, and separated using olfaction techniques and TLC. First, odorants will be separated using TLC, and the components of an unknown odorant mixture will be identified through calculating their Rf values. Second, your sense of smell will be used to identify odorants and determine olfaction and detection thresholds.

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is for students to understand that olfactory receptors respond to smells and transmits them as signals to the brain. Students will also be able to understand the principles of thin layer chromatography and how they apply to separation of olfactory compounds.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Module I:
Thin Layer Chromatography - 45 min.

Module II:
Detection of Scents - 20 min.
(Can be completed while the TLC plate is incubating in the solvent.)

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

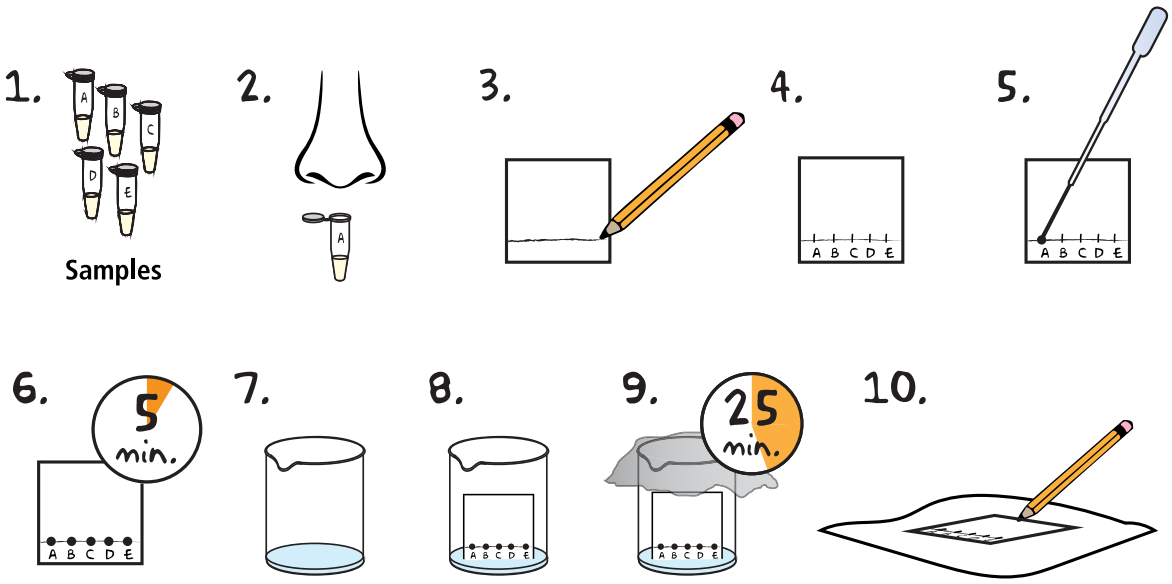
During the Experiment:

- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Module I: Thin Layer Chromatography

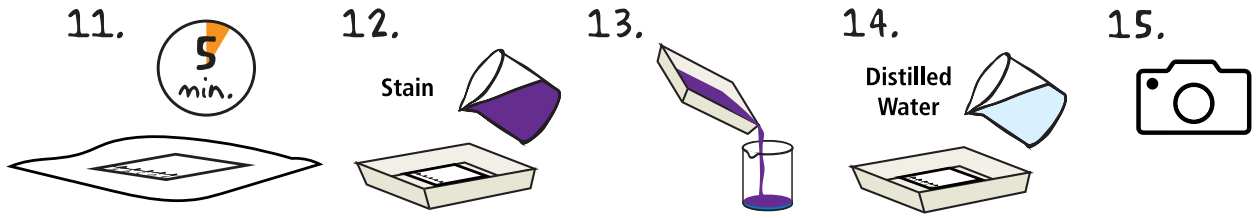


Tube A - Limonene
Tube B - Anethole
Tube C - Eugenol
Tube D - S-Citronellal
Tube E - Unknown

1. **OBTAIN** the sample tubes A-E from your instructor.
2. **SMELL** sample tubes A-D and the unknown, tube E. Make a hypothesis as to the components of tube E. **RECORD** your hypothesis.
3. **OBTAIN** 1 TLC plate from teacher. **MEASURE** 1 cm from the bottom (longer) edge and very gently **DRAW** a straight line across using a blunt (unsharp) pencil. This will serve as the origin line.
   NOTE: Be sure to use a regular pencil, not a pen or colored pencil. The ink in a pen will move with the samples during the experiment and contaminate results. Additionally, write VERY SOFTLY on the TLC plate as not to tear the polymer.
4. Using a blunt pencil, gently **DIVIDE** the origin line into 5 spots starting 0.5 cm from the edge and leaving 1.5 cm between each spot. **MARK** the location in the line and the sample letter you will be spotting with a pencil.
5. Use a transfer pipet to **TRANSFER** 1 drop of sample A into spot A. Take care to deposit only ONE drop, a very small amount of compound. Very little sample (~1 µL) is necessary to achieve results. Make the diameter of the spot as small as possible.
6. **REPEAT** step 4 for samples B-E. Let the sample spots **DRY** at room temperature for 5 min.
7. **TRANSFER** 10 mL of solvent into a beaker. This should be fill the beaker approximately 0.5 cm with solvent.
8. **PLACE** the bottom edge of the TLC plate (with the spots) into the beaker and lean it up against the side so that it remains upright.
9. **COVER** the container with saran wrap to prevent solvent evaporation. Let the plate stand in the solvent for 35 minutes. The solvent front should move up the TLC plate, but not reach the top of the plate in this time.
10. **REMOVE** the TLC plate and lay it flat (adsorbent side up) on a paper towel. **MARK** the edge of the solvent front with a pencil.

continued

Module I: Thin Layer Chromatography, continued



11. **ALLOW** the TLC plate to dry at room temperature for 5 minutes.
12. **MOVE** the TLC plate to a clean container. **POUR** staining solution into the container, and **GENTLY ROCK** twice to be sure the whole plate gets covered. ***NOTE: Be sure to wear gloves as the stain can stain hands and clothing.***
    - Alternatively, the TLC plate can be dipped into a beaker containing the staining solution. Just be sure that the entire TLC plate is covered.
13. **POUR** the staining solution back into the beaker.
14. **WASH** the plate with distilled water until there is no more purple coming off the plate.
15. **TAKE A PHOTO** of the plate immediately for analysis. As the plate dries, the color may change, but the dark sample spots should remain the same. The plate will be completely dry after 24 hours and can be taped into a lab notebook or re-analyzed.


Wear gloves and safety goggles

EVALUATE THE SAMPLES

16. **MEASURE** the distance that the solvent front traveled from the origin point. **RECORD** this value in Table 2.
17. **MEASURE** the distance that samples A-D traveled from the origin point. **RECORD** this value in Table 2. When identifying how far a compound traveled, measure from the center of the spot. Additionally, if a compound has more than one Rf value, measure both spots.
18. **CALCULATE** the Rf value of samples A-D. The Rf value is the distance traveled by a sample from its origin divided by the distance that the solvent traveled.
19. **IDENTIFY** the number of distinct compounds that were separated in sample E. **MEASURE** the distance that each point in sample E traveled. **RECORD** these values in Table 2.
20. **CALCULATE** the Rf value of each component of sample E. Using the Rf components calculated in step 3, and the Rf value of each component in sample E, identify the components of sample E.
21. **DETERMINE** if your hypothesis about the identity of sample E was correct.

TABLE 2	Distance Travelled	Rf
Solvent Front		N/A
Sample A		
Sample B		
Sample C		
Sample D		
Sample E (Unknown)		

Module II: Detection of Scents

1. **OBTAIN** the Unknown 1 dilution series (5 tubes), Unknown 2 dilution series (5 tubes), and water control (1 tube).
2. **SMELL** the distilled water control tube first and then **SMELL** the Unknown 1 dilution series starting with Tube 1-5 and ending with Tube 1-1.
3. **SCORE** the strength of the odor from 1-10, with 1 being the weakest and 10 being the strongest.
4. **RECORD** your score in Table 1 below.
5. **NOTICE** if you can identify the smell. If you are able to identify the smell, **RECORD** which tube number you recognize it at.
6. **REPEAT** steps 2 through 5 with Unknown 2 dilution series starting with Tube 2-5 and ending with Tube 2-1 (most dilute to most concentrated).

TABLE 1		Tube 1-1	Tube 1-2	Tube 1-3	Tube 1-4	Tube 1-5
Unknown 1	Volume Compound (µL)	8	0.8	0.08	0.008	0.0008
	Volume Water (µL)	900	900	900	900	900
	Odor Strength					
		Tube 2-1	Tube 2-2	Tube 2-3	Tube 2-4	Tube 2-5
Unknown 2	Volume Compound (µL)	0.5	0.05	0.005	0.0005	0.00005
	Volume Water (µL)	900	900	900	900	900
	Odor Strength					

7. **CALCULATE** the threshold odor number individually based on the tube at which you were able to identify the odor. See Table 1 (above) for the volume of odor causing sample and the volume of odor free water.

Threshold Odor Number (TON) refers to the amount of substance required to be detected.
It is calculated using the formula:

$$TON = A + (B/A)$$

A = the volume of the odor causing sample, and B = the volume of odor-free water.

8. **ANALYZE** the class data to find the average threshold odor number.
9. Using the class data for which concentration students were able to identify the odor, **CALCULATE** the odor recognition threshold. The odor recognition threshold refers to the concentration of an odor whereby it can be recognized by 50% of individuals.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. How are we able to detect smells?
2. How can we quantify a smell?
3. What are the basic principles of Thin Layer Chromatography?

Instructor's Guide

NOTES TO THE INSTRUCTOR

This lab is designed for 10 lab groups. Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

Preparation For:	What to do:	When:	Time Required:
Module I: Thin Layer Chromatography	Dilute odorants	Up to 1 day before performing the lab.	10 min.
	Prepare reagents	Anytime before performing the lab.	10 min.
Module II: Detection of Scents	Prepare and dilute odorants	Up to 1 day before performing the lab.	20 min.

Pre-Lab Preparations

MODULE I

Dilute Odorants

1. **ADD** 1 mL Ethanol to Component A, Limonene. **MIX** well.
2. **ADD** 1 mL Ethanol to Component B, Anethole. **MIX** well.
3. **ADD** 1 mL Ethanol to Component C, Eugenol. **MIX** well.
4. **ADD** 1 mL Ethanol to Component D, S-Carvone. **MIX** well.
5. **ADD** 1 mL Ethanol to Component E, unknown sample. **MIX** well.

NOTE: Save Limonene and Anethole to make dilutions in Module II.

Prepare Reagents

6. **CUT** the TLC paper into 6 x 7 cm plate using scissors. Be sure to handle the plate by its edges and be gentle so the polymer doesn't peel off. Each group will get one piece of TLC paper.
7. **ALIQUOT** 10 µL of Components A-E for each group.
8. **DISTRIBUTE** 5 transfer pipets per group.
9. **PREPARE** solvent: 40% ethanol. For 100 mL, **MIX** 40 mL ethanol and 60 mL water. Depending on the size of the beakers or cups used for chromatography, you may need more or less of the solvent. The solvent should fill to a height of 0.5 cm from the bottom of the cup or beaker.
10. **PREPARE** staining solution by dissolving the anhydrous sodium carbonate in a beaker with 100 mL water. Once dissolved, **ADD** the potassium permanganate and **MIX** well. **WRAP** the beaker with foil to protect the solution from light. The solution can be placed at a table in the classroom for students to share.

Pre-Lab Preparations

MODULE II

Preparation and Dilution of Odorants

Use the provided Limonene (A) and Anethole (B) to make serial dilutions of 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000. Each group will receive 10 tubes: 5 for dilution series 1 (Limonene) and 5 for dilution series 2 (Anethole).

1. Label 5 tubes 1-1, 1-2, 1-3, 1-4, 1-5 and add 900 µL distilled water to each. (These will be used for Limonene.)

2. Label 5 tubes 2-1, 2-2, 2-3, 2-4, 2-5 and add 900 µL distilled water to each. (These will be used for Anethole.)

Note: The odorants are provided in ethanol, but you will be diluting them with water. They will precipitate and form a cloudy mixture. Take care to shake or vortex well before performing the dilutions.

3. Transfer 100 µL Limonene to tube 1-1, pipette the solution up and down, cap the tube, and mix or vortex well.

4. Transfer 100 µL sample from tube 1-1 to tube 1-2, pipette the solution up and down, cap the tube and mix well.

5. Transfer 100 µL sample from tube 1-2 to tube 1-3, pipette the solution up and down, cap the tube, and mix or vortex well.

6. Transfer 100 µL sample from tube 1-3 to tube 1-4, pipette the solution up and down, cap the tube, and mix or vortex well.

7. Transfer 100 µL sample from tube 1-4 to tube 1-5, pipette the solution up and down, cap the tube and mix or vortex well.

8. Aliquot 100 µL from tubes 1-1 through 1-5 for 10 groups.

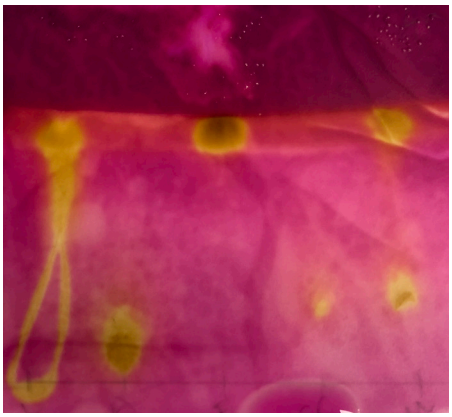
9. Repeat steps 3 through 8 for Anethole, using tubes labeled 2-1 through 2-5.

10. Aliquot 100 µL from tubes 2-1 through 2-5 for 10 groups.

11. Aliquot 100 µL of distilled water for each group.

Expected Results and Analysis

Module I



A B C D E

Lane	Sample	Rf value
A	Limonene	-0.02, 0.7
B	Anethole	0.125
C	Eugenol	1
D	S-Citronellal	0.3
E	Unknown*	0.3, 1

**Unknown sample in Module I is S-citronellal and Eugenol.*

Module II

Each class will likely have different odor detection and threshold odor numbers. Unknown sample 1 is Limonene, and commonly recognized as a lemon smell. Unknown sample 2 is Anethole, and is commonly recognized as anise, fennel, licorice, and magnolia blossoms.

Please refer to the kit insert for the Answers to Study Questions

Appendix A

Substituting Isopropanol for Ethanol

Using ethanol for the Dilution of Odorants and the solvent (Module I), is ***highly preferred***. However, isopropanol can be substituted if ethanol is unavailable. There are slight disadvantages associated with using isopropanol:

- Separation of the components in Module I is less clear.
- Strong scent may inhibit identification of the components in Module II.

Module I: Dilution of Odorants (replaces steps 1-5, page 13)

1. Add 100 µL isopropanol to Component A, Limonene. Mix well.
2. Add 500 µL isopropanol to Component B, Anethole. Mix well.
3. Add 500 µL isopropanol to Component C, Eugenol. Mix well.
4. Add 500 µL isopropanol to Component D, S-Carvone. Mix well.
5. Add 500 µL isopropanol to Component E, unknown sample. Mix well.

Module I: Solvent Preparation (replaces step 9, page 13)

- Mix 35 mL isopropanol with 65 mL water to create 35% isopropanol solvent.