

## Chlorophyll Extraction

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### - 1 - Introduction

Leaves contain various pigments, such as green chlorophyll, yellow-orange carotenoids, and red anthocyanins. By extracting the pigments from the leaves and separating them using thin-layer chromatography (TLC), these pigments can be observed. This experiment demonstrates how the pigments differ in solubility and mobility.

This experiment shows that leaves consist of various pigments with different colors. Thin-layer chromatography allows these pigments to be separated and visualized.

Let's get started and have some fun with science!

### - 2 - Materials

Material		<i>Alternatives</i>
Fresh green leaves	e.g. spinach, dandelion or parsley	
Mortar and pestle		
Aceton		You may use ethanol (> 70%)
Thin-layer chromatography (TLC) plate		You may use a coffee filter paper
Chromatography solvent	8 ml petroleum ether + 2 ml acetone	You may use gas instead of petroleum
Beaker or glass container with a lid		You may use a jam jar
Filter paper		You may use paper from a kitchen roll
Tweezers		
Pipette or glass rod		
Protection	Protective gloves, Safety goggles, Lab coat	

## - 3 - Experiment

### Extraction of the Leaf Pigments

- Cut fresh green leaves into small pieces.
- Place the leaf pieces in a mortar and add a small amount of acetone.
- Use the pestle to grind the leaves thoroughly until you obtain a green solution.
- The addition of sea sand can enhance the extraction.
- Filter the green solution through filter paper into a small glass to remove solid plant debris.
- You may want to evaporate a part of the chlorophyll solution in the dark to gain a higher concentration.



### Prepare the Chromatography Plate:

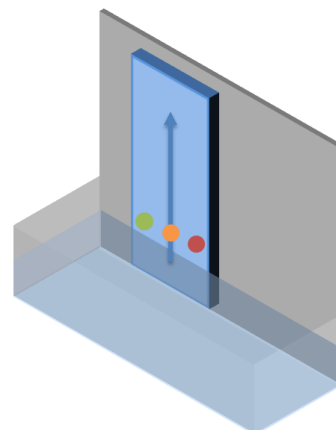
- Cut the TLC plate to fit inside your glass container (developing chamber).
- Draw a thin pencil line (starting line) 1–2 cm from the bottom edge of the TLC plate.
- Use a pipette to carefully apply a small drop of the extracted pigment solution onto the starting line of the TLC plate. Let the drop dry and repeat this process to concentrate on the pigment spot.

### Prepare the Chromatography Solvent:

- Pour a small amount of chromatography solvent (8 ml petroleum ether and 2 ml acetone) into the beaker or glass container to a depth of about 1 cm.
- Ensure that the solvent does not touch the pigment spot directly.

### Run the Chromatography:

- Place the TLC plate vertically into the beaker, ensuring the solvent reaches the bottom of the plate but not the pigment spot.
- Cover the beaker with a lid to prevent evaporation and allow the solvent to travel up the TLC plate.
- Once the solvent front approaches the top of the plate (after about 15–30 minutes), remove the plate from the beaker and mark the solvent front (solvent line) with a pencil.
- Let the TLC plate dry in the air.



### Analyze the Results:

- Examine the different colored bands on the TLC plate. Each band represents a different pigment in the leaf, which is separated based on its solubility and interaction with the TLC plate.
- Common pigment colors include green (chlorophyll), yellow (carotenoids), and orange (xanthophylls).

## - 4 - Additional Information & Safety Instructions

All individuals conducting the experiments outlined in this protocol must thoroughly review and adhere to all safety instructions and guidelines. It is imperative that each person reads the Material Safety Data Sheets (MSDS) for every chemical involved prior to commencing any experiment. Failure to follow proper safety procedures can result in serious injury or harm. The responsibility for ensuring a safe working environment lies with each individual participant. The author and distributor of this protocol assume no liability for accidents, injuries or damages resulting from the misuse of the information provided.

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### Important Safety Notices:

- Wear safety goggles and gloves to protect your eyes and skin.
- Handle acids, ethanol and petroleum with care.
- Perform the experiment in a well-ventilated area or under a fume hood.
- Dispose of chemicals properly after the experiment.
- Read and follow the MSDS for all chemicals