



Determining Chlorophyll Concentration using CuSO4 Magnesium-Copper Exchange Titration

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ARSTRACT

This protocol is designed to be able to extract and analyze the concentration of chlorophyll within a sample on a molecular level. The procedures of this protocol require using copper II sulfate and hydrochloric acid as a means of trituration to determine the approximate level a chlorophyll within a given sample.

GUIDELINES

For proper extraction in titration of Chlorophyll concentration you must be able to measure and transfer liquids within a hundred microliters ensure that samples are separated and free of contaminants.

MATERIALS

NAME ~	CATALOG # ~	VENDOR ~	
Magnesium sulfate heptahydrate			
Hydrochloric Acid			
Copper (II) sulfate pentahydrate	CDB0063.SIZE.500g	Bio Basic Inc.	
Acetone	00310-95	Nacalai Tesque	
Distilled Water	15230196	Thermo Fisher	
STEPS MATERIALS			
NAME V	CATALOG # V	VENDOR ~	
Magnesium sulfate heptahydrate			
Magnesium sulfate heptahydrate			
Acetone	00310-95	Nacalai Tesque	
Acetone	00310-95	Nacalai Tesque	
Copper (II) sulfate pentahydrate	209198	Sigma - Aldrich	
Hydrochloric Acid			
Acetone	00310-95	Nacalai Tesque	

SAFETY WARNINGS

This protocol requires the use of strong corrosive acids, corrosive and flammable solvents, and require the extraction of pigments that may stain clothing. Proper lab coat, eye protection, gloves in ventilation are required to conduct is chlorophyll extraction in titration protocol. Also, care must be taken to ensure that all materials used are disposed properly, as many of the chemicals may be hazardous to health and environment.

BEFORE STARTING

In order to perform this chlorophyll extraction protocol you will need the following materials and chemicals:

the materials listed are based on one single sample, in must be multiplied based on the number of samples you would like to test

Two 20 ml (minimum) test tube

Ten 5 ml glass vials

A minimum of ten 200 micro-liters PCR tubes (although more may be needed based on the accuracy necessary for the procedures performed)

A 100 to 1000 micro-liter adjustable pipette

30 disposable 1000 micro-liter pipette tips (Number varies based on need and mistakes)

1200 g Centrifuge

One 20 micron filtration filter paper

Mortar and Pestle

Transfer Pipettes (as needed for contamination prevention)

10 ml Graduated Cylinder

Test tube stirrers

Approximate protocol time: 6 hours total - 3 hours Preparation - 3 Hours Sample Sit time

100ul per PCR Tube Copper II sulfate Solution 60mg/ml Distilled H20

As needed Distilled water

9 ml Acetone

1g Magnesium sulfate

2g of sample

Preparation of Sample and Extraction

Add 22 g of sample plant matter or other test article to a mortar

1m

1m

2 Add **1** g of



crystals to the mortar.

5m

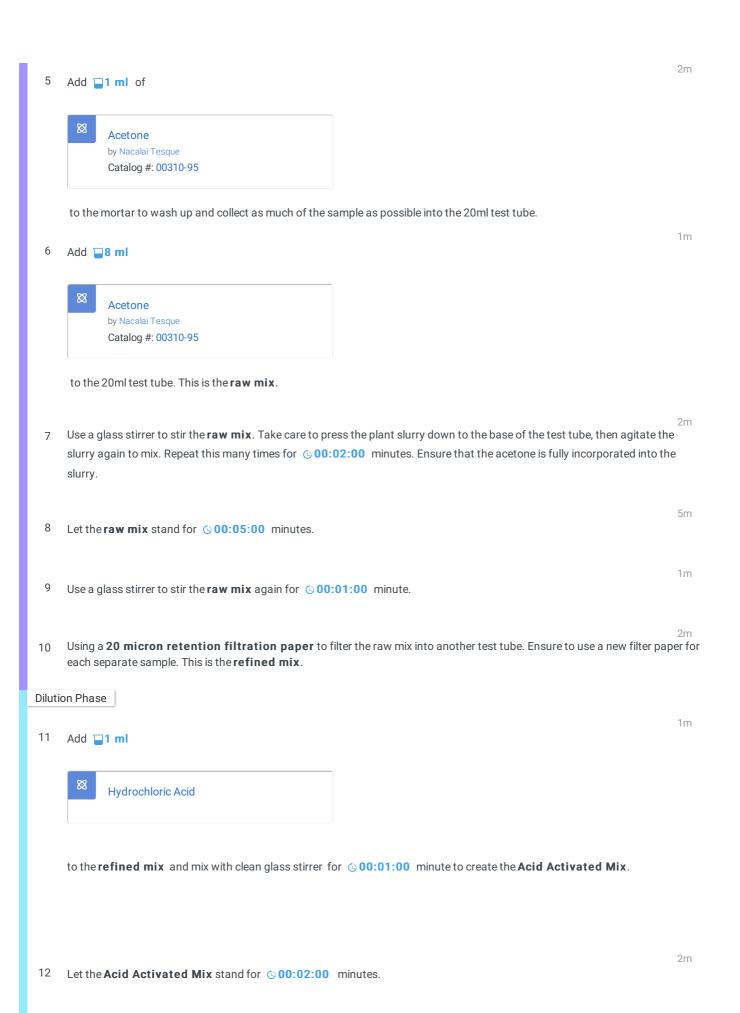
3 Grind the sample item and

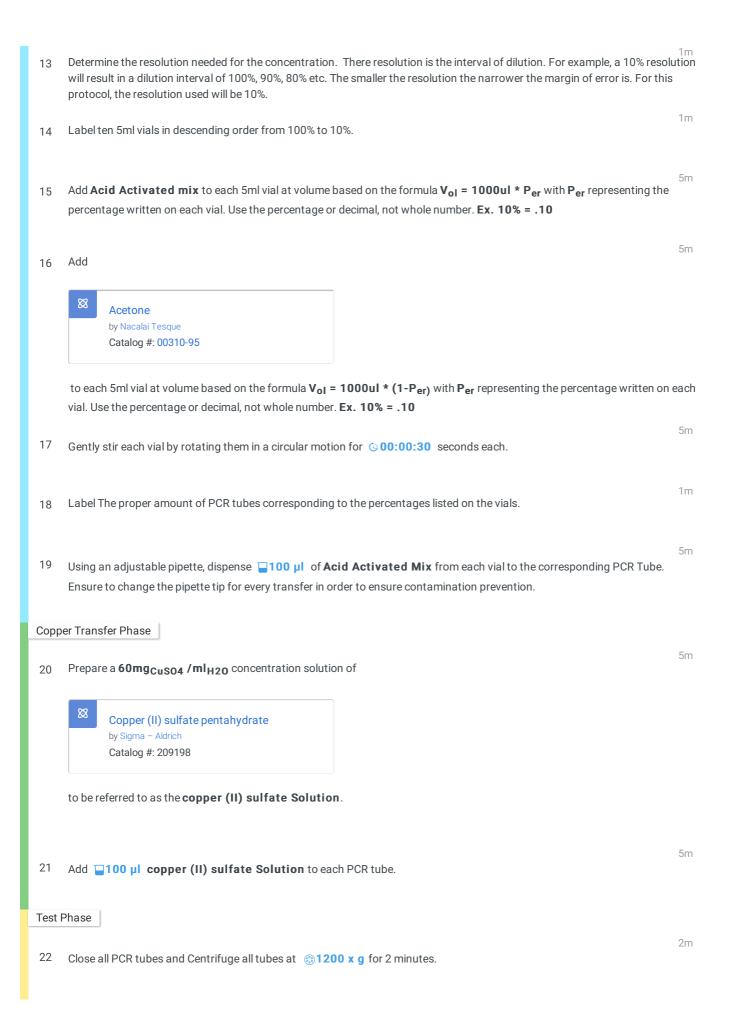


together until the leaves are ground up, the magnesium sulfate is completely dissolved into the plant liquid and a liquid starts to form.

4 Transfer as much of the solid and liquid slurry to a 20ml test tube.

1m





2m

24 Centrifuge all tubes at **31200** x g for 2 minutes.

Results Phase

1m

- Observe which PCR is the lowest percentage concentration not to have any trace of precipitated **Copper (II) Sulfate**. (A blue crystalline precipitate at the bottom. Take note of the percentage number to be used as the variable **P**₋.
 - 1m
- 26 Assign the highest percentage value vial to have visible copper (II) Sulfate precipitate the variable P+.
- 10m

27 Use this formula to calculate the mean value for the concentration in g/ml.

$$(6*10^{-4}*893.51)/(P_-*159.609)+(((6*10^{-4}*893.51)/(P_+*159.609) - (6*10^{-4}*893.51)/(P_-*159.609))/2)=$$
 Con

10m

28 Use this formula to calculate the margin of error of the concentration.

$$((6*10^{-4}*893.51)/(P_+*159.609) - (6*10^{-4}*893.51)/(P_-*159.609))/2 = Moe$$

1m

29 Write the final results as Con ± Moe

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