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Determining Chlorophyll Concentration using CuSO4 Magnesium-Copper Exchange Titration

Victor Rodriguez[1]

[1]Independent Researcher

1 Works for me dx.doi.org/10.17504/protocols.io.7xrhpm6

Victor Rodriguez

ABSTRACT

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 micro-liters 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	CATALOG #	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 H2O
As needed Distilled water
9 ml Acetone
1g Magnesium sulfate
2g of sample

Preparation of Sample and Extraction

1m

1 Add **2 g** of sample **plant matter** or other test article to a mortar

.

1m

2 Add **1 g** of

Magnesium sulfate heptahydrate

crystals to the mortar.

5m

3 Grind the sample item and

Magnesium sulfate heptahydrate

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

1m

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

2m

5 Add **1 ml** of

Acetone
by Nacalai Tesque
Catalog #: 00310-95

to the mortar to wash up and collect as much of the sample as possible into the 20ml test tube.

1m

6 Add **8 ml**

Acetone
by Nacalai Tesque
Catalog #: 00310-95

to the 20ml test tube. This is the **raw mix**.

2m

7 Use a glass stirrer to stir the **raw mix**. Take care to press the plant slurry down to the base of the test tube, then agitate the slurry again to mix. Repeat this many times for **00:02:00** minutes. Ensure that the acetone is fully incorporated into the slurry.

5m

8 Let the **raw mix** stand for **00:05:00** minutes.

1m

9 Use a glass stirrer to stir the **raw mix** again for **00:01:00** minute.

2m

10 Using a **20 micron retention filtration paper** to filter the raw mix into another test tube. Ensure to use a new filter paper for each separate sample. This is the **refined mix**.

Dilution Phase

1m

11 Add **1 ml**

Hydrochloric Acid

to the **refined mix** and mix with clean glass stirrer for **00:01:00** minute to create the **Acid Activated Mix**.

2m

12 Let the **Acid Activated Mix** stand for **00:02:00** minutes.

1m

13 Determine the resolution needed for the concentration. There resolution is the interval of dilution. For example, a 10% resolution will result in a dilution interval of 100%, 90%, 80% etc. The smaller the resolution the narrower the margin of error is. For this protocol, the resolution used will be 10%.

1m

14 Label ten 5ml vials in descending order from 100% to 10%.

5m

15 Add **Acid Activated mix** to each 5ml vial at volume based on the formula $\mathbf{V_{ol} = 1000ul * P_{er}}$ with $\mathbf{P_{er}}$ representing the percentage written on each vial. Use the percentage or decimal, not whole number. **Ex. 10% = .10**

5m

16 Add

Acetone
by Nacalai Tesque
Catalog #: 00310-95

to each 5ml vial at volume based on the formula $\mathbf{V_{ol} = 1000ul * (1\text{-}P_{er})}$ with $\mathbf{P_{er}}$ representing the percentage written on each vial. Use the percentage or decimal, not whole number. **Ex. 10% = .10**

5m

17 Gently stir each vial by rotating them in a circular motion for **00:00:30** seconds each.

1m

18 Label The proper amount of PCR tubes corresponding to the percentages listed on the vials.

5m

19 Using an adjustable pipette, dispense **100 µl** of **Acid Activated Mix** from each vial to the corresponding PCR Tube. Ensure to change the pipette tip for every transfer in order to ensure contamination prevention.

Copper Transfer Phase

5m

20 Prepare a $\mathbf{60mg_{CuSO4}\ /ml_{H2O}}$ concentration solution of

Copper (II) sulfate pentahydrate
by Sigma – Aldrich
Catalog #: 209198

to be referred to as the **copper (II) sulfate Solution**.

5m

21 Add **100 µl** **copper (II) sulfate Solution** to each PCR tube.

Test Phase

2m

22 Close all PCR tubes and Centrifuge all tubes at **1200 x g** for 2 minutes.

6h

23 In a PCR rack, let the samples stand for 6 hours

2m

24 Centrifuge all tubes at **1200 x g** for 2 minutes.

Results Phase

1m

25 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**