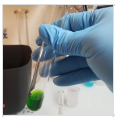

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# Chlorophyll Extraction and Spectral Analysis with Spectrometer Calibration V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.8eahtae

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ABSTRACT

This protocol is designed to be able to extract and analyze the concentration of chlorophyll within a sample of a given plant. The procedures of this protocol require using a spectroscope to determine the approximate level a chlorophyll within a given sample.

GUIDELINES

For proper extraction and spectroscopy 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, ACS Grade | M-020 | Gold Biotechnology |
| Acetone | 34850 | Sigma Aldrich |

STEPS MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Magnesium sulfate, heptahydrate, ACS | MB0329.SIZE.2.5Kg | Bio Basic Inc. |
| Acetone | 34850 | Sigma Aldrich |
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| Acetone | 34850 | Sigma Aldrich |
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| Acetone | AC1200.SIZE.1L | Bio Basic Inc. |

SAFETY WARNINGS

This protocol requires the use of flammable solvents, and require the extraction of pigments that may stain clothing. Proper lab coat, eye protection, gloves in ventilation are required to conduct this chlorophyll extraction and concentration 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

One 20 ml (minimum) test tube
40 ml beaker
Two 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

5 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)
50 ml Graduated Cylinder
Test tube stirrers
Approximate protocol time: 1 hours total
As needed Distilled water
15 ml Acetone
0.13g Magnesium sulfate
0.25g of sample
Spectrometer

## Extraction of Chlorophyll

1 Weigh out **0.25 g** of sample subject and add it to a pestle. 1m

2 Weigh out **0.13 g** of 1m

Magnesium sulfate, heptahydrate, ACS
by Bio Basic Inc.
Catalog #: MB0329.SIZE.2.5Kg

and add it to a pestle.

3 Add **1 ml** of 1m

Acetone
by Sigma Aldrich
Catalog #: 34850

to the pestle.

4 Grind the entire mixture until the sample is consistent and the 5m

Magnesium sulfate, heptahydrate, ACS
by Bio Basic Inc.
Catalog #: MB0329.SIZE.2.5Kg

is completely dissolved into the sample paste.

5 Add the paste into a 40 ml beaker and wash out the pestle with **1 ml** of 1m

Acetone
by Sigma Aldrich
Catalog #: 34850

and empty it into the beaker.

6 Add **13 ml** of 2m

Acetone
by Sigma Aldrich
Catalog #: 34850

to the 40 ml beaker and mix the sample thoroughly, ensuring to press and remix the sample several times.

7 Let the sample beaker stand for 15 minutes. 15m

8 Filter the sample into a 20 ml test tube using a 20 micron retention filter paper. 1m

9 Let the sample stand for a further 10 minutes. 10m

## Sample Analyzation

10 Add **200 µl** of the sample to two PCR tubes and label them according to your needed labeling system. the two tubes will serve as double tests for verification. 1m

11 Centrifuge the two samples to 1200 g to settle out any particulates for 1 minute. 1m

12 From each PCR tube, transfer **100 µl** of the sample from the top to another PCR Tube. 2m

13 Add **100 µl** of

Acetone
by Bio Basic Inc.
Catalog #: AC1200.SIZE.1L

to a PCR tube and label it "base"

14 Use the "base" PCR tube to calibrate the spectrometer.

15 Place each tube in the spectrometer. 1m

16 Take to log base 10 of the absorbance percentage of the wavelength 647 nm and 664.5 nm and denote them as $A_{647}$ and $A_{664}$. 5m

17 To calculate the concentration... 5m

$CHL_A = 20.47A_{647} - 4.73A_{664}$
$CHL_B = 12.63A_{664} - 2.52A_{647}$
$CHL_{Total} = CHL_A + CHL_B$

Divide each concentration by 1000, multiply by the acetone used, then divide by the sample mass to get the mg/g of sample

Inskeep, William P., and Paul R. Bloom. (1985). Extinction Coefficients of Chlorophyll a and b in N,N-Dimethylformamide and 80% Acetone.. Plant Physiology, vol. 77, no. 2, Jan. 1985. http://doi:10.1104/pp.77.2.483

18 Confirm readings match to within an acceptable margin of error. 5m