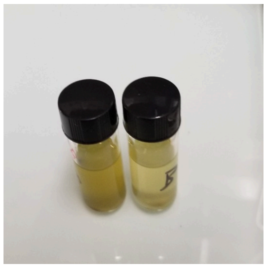
Oct 19, 2019

Starch Concentration Protocol



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Protocol status: Working
We use this protocol and it's working

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Abstract

The aim of this protocol is to extract and analyze the starch content of a plant in order to gain data into its metabolism and photosynthetic properties.

Guidelines

For proper extraction and spectroscopy of starch 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

MATERIALS

Acetone **Bio Basic Inc.** **Catalog #**AC1200.SIZE.1L

Iodine Solution 0.9% Aldon Corporation **Catalog #**IS18019

STEP MATERIALS

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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 starch 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 start

In order to perform this starch 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
200 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
A 0.5 to 10 micro-liter adjustable pipette
5 disposable 1000 micro-liter pipette tips (Number varies based on need and mistakes)
5 disposable 10 micro-liter pipette tips (Number varies based on need and mistakes)
1200 g Centrifuge
Mortar and Pestle
Transfer Pipettes (as needed for contamination prevention)
50 ml Graduated Cylinder
Test tube stirrers
Approximate protocol time: 3 hours total
As needed Distilled water
15 ml Acetone
0.25g of sample
Spectrometer
Autoclave

Pigment Extraction

1 Weigh out .25 g of plant sample to be tested and add it to a 200ml beaker. 1m

2 Add 20 mL of
Acetone **Bio Basic Inc. Catalog** #AC1200.SIZE.1L
to the test tube. 1m

3 Let the sample/acetone suspension sit in a 200 ml beaker filled with distilled water and heat water until the of
Acetone **Bio Basic Inc. Catalog** #AC1200.SIZE.1L
starts to boil. 10m

4 Let test tube cool for 00:30:00 minutes to extract the pigments of the plant. 30m

5 Drain the
Acetone **Bio Basic Inc. Catalog** #AC1200.SIZE.1L
and add 20 mL more
Acetone **Bio Basic Inc. Catalog** #AC1200.SIZE.1L
and put it back into the beaker heat water until the of
Acetone **Bio Basic Inc. Catalog** #AC1200.SIZE.1L
starts to boil. 1m

6 Let test tube cool for 00:30:00 minutes more to finish extracting the pigments. 30m

7 Drain the test tube and transfer the sample to an autoclave for heating for 01:00:00 hour at 135 °C . This will loosen up the starch for extraction. 1h

8 Remove the sample from the autoclave. 1m

9 Grind the sample with a mortar and pestle until a paste. 5m

10 Add 5 mL of distilled water to the pestle and wash around to dissolve the sample into a suspension and clean the pestle. Ensure all of the sample is in the suspension. 5m

11 Funnel the suspension into a test tube and add an additional 5 mL of distilled water to the test tube using the same funnel as to wash out the funnel of stuck on sample particles. 1m

12 Let stand for an hour covered. 1m

13 Add 3 mL of the suspension to a 3 ml vial and 1 mL of the suspension to another 3 ml vial. Mark the vials as needed for the protocol. Add 2 mL of distilled water to the vial with 1ml. This will serve as a 1/3 diluted sample in case the concentration is too high for the spectrometer to read the original sample. 2m

14 To two labeled PCR tubes, add 200 µL from each vial and centrifuge for 00:01:00 at 1200 x g .

15 Take 100 µL from each PCR tube and transfer it to clean PCR tubes labeled. Ensure that the sample is removed from the top to prevent sucking up settled particulates. 1m

16 Add 100 µL of distilled water to a PCR tube and calibrate the Spectrometer with it. 1m

17 Insert each sample into the spectrometer. 5m

18 The Absorbance of the samples are to be taken at the 610nm wavelength and the log base 10 of the absorbance percentage is noted. 1m

19 Use the following function to determine the concentration... 5m

$C_{st} = (A_{610} * 692.7_{g/mol} * V_{ml)} / (2594 * M_{sm})$

With C_{st} being the starch concentration in mg/g, A_{610} is the absorbance at 610 nm, V_{ml} is the volume in ml of the water added to the sample for extraction, and M_{sm} is the mass in grams of the original sample taken.