



Total Starch Enzymatic Digestion V.2

COMMENTS 0

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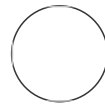
¹Realizing Increased Photosynthetic Efficiency (RIPE)

VERSION 2
DEC 09, 2022

Burgess Lab UIUC

WORKS FOR ME

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Lynn Doran

Realizing Increased Photosynthetic Efficiency (RIPE)

ABSTRACT

Enzymatic digestion of total soluble starch to glucose in plant tissue extracts for preparation for quantification via the GOD-POD Method (NZYtech).

PROTOCOL CITATION

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<https://protocols.io/view/total-starch-enzymatic-digestion-cj9qur5w>
Version created by Lynn Doran

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Pak S. Chow, Simon M. Landhäusser, A method for routine measurements of total sugar and starch content in wood plant tissues, Tree Physiology, Volume 24, Issue 10, October 2004, Pages 1129–1136,
<https://doi.org/10.1093/treephys/24.10.1129>

KEYWORDS

Starch, Soluble Starch, Enzymatic Digestion, Plant Tissue, GOD-POD

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CREATED

Dec 08, 2022

LAST MODIFIED

Dec 09, 2022

MATERIALS TEXT

Reagents

- α -amylase enzyme, 1000 U/mL
- Amyloglucosidase enzyme, 3260 U/mL
- MOPS Buffer 10mM, pH 6.5

 2.09 g of MOPS per  1 L of water ( 0.4598 g for  220 mL water), pH adjusted to 6.5 using NaOH.

Store refrigerated for up to 2 months.

- Acetate Buffer 100mM , pH 4.5

 6 mL of acetic acid per  1 L of water ( 1.5 mL per  250 mL water), pH adjusted to 6.5 using NaOH.

Store refrigerated for up to 2 months.

- Ice

Materials

- Pipette tips

Equipment

- Graduated cylinder
- Water bath
- Floating tube holder
- Single channel pipette
- Ice bucket

BEFORE STARTING






Extract and dry total starch pellet from plant tissue per [Extraction of Non-Structural Carbohydrates \(Total Soluble Sugars + Starch\) in Plant Tissues](#).

- 1 Prepare fresh daily 120 U/mL α -amylase in MOPS buffer. 1 mL per sample will be needed. Initial concentration of α -amylase is 1000 U/mL. Use $C_1V_1 = C_2V_2$ to calculate the volume of α -amylase and MOPS buffer to use.

Note

- 2 Prepare fresh daily 30 U/mL amyloglucosidase in acetate buffer. 1 mL per sample will be needed. Initial concentration of amyloglucosidase is 3260 U/mL. Use $C_1V_1 = C_2V_2$ to calculate the volume of amyloglucosidase and acetate buffer to use.


Note

- 3 Heat the water bath to  75 °C .
- 4 Add  500 µL of α-amylase in MOPS buffer (120 U/mL) to each sample tube.
- 5 Vortex to suspend all solids. Flicking the tube may help dislodge the dried starch pellet.
- 6 Incubate for  00:30:00 at  75 °C in the water bath. 30m
- 7 Add another  500 µL of α-amylase in MOPS buffer (120 U/mL) to each sample tube.


8 Vortex to suspend all solids.

9 Incubate for  00:30:00 at  75 °C in the water bath.



30m

10 Cool down the water bath to  50 °C, tubes can be stored at room temp on the counter while water bath cools.


Note

11 Add  500 µL amyloglucosidase in acetate buffer (30 U/mL) to each sample tube.



12 Vortex to suspend all solids.

13 Incubate for  00:30:00 at  50 °C in the water bath.

30m

14 Add another  500 µL amyloglucosidase in acetate buffer (30 U/mL) to each sample tube.

15 Vortex to suspend all solids.

- 16 Incubate for  00:30:00 at  50 °C in the water bath.
- 17 Place the tubes in ice to stop the reaction, until cool to the touch.
- 18 Proceed to total starch (as glucose) quantification by NZYtech GOD-POD method or store the samples at -20°C up to one month.