Total Starch Enzymatic Digestion V.2

This protocol is published without a DOI.

Lynn Doran[1], Amanda P. De Souza[1]

[1]Realizing Increased Photosynthetic Efficiency (RIPE)

Burgess Lab UIUC

Lynn Doran
Realizing Increased Photosynthetic Efficiency (RIPE)

VERSION 2
DEC 09, 2022

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

Lynn Doran, Amanda P. De Souza 2022. Total Starch Enzymatic Digestion . **protocols.io** 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 woody 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

CREATED

Dec 08, 2022

LAST MODIFIED

Dec 09, 2022

PROTOCOL INTEGER ID

73744

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.

30m

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

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.

30m

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.