Total Starch Enzymatic Digestion V.3

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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ABSTRACT

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

MATERIALS

Reagents

- α-amylase (Bacillus licheniformis) enzyme, 3000 U/mL
- Amyloglucosidase (Aspergillus niger) 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

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

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BEFORE START INSTRUCTIONS

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 3000 U/mL. Use $C_1V_1 = C_2V_2$ to calculate the volume of α-amylase and MOPS buffer to use.

Note

α-amylase comes at 3000 U/mL concentration in a 20 mL bottle. If using all of the bottle, 20 mL of α-amylase in 146.7 mL of MOPS will result in 120 U/mL α-amylase in MOPS buffer.

To prepare 250 mL of 120 U/mL α-amylase in MOPS buffer, dilute 10 mL α-amylase (3000 U/mL) into 240 mL MOPS buffer.

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

To prepare 250 mL of 30 U/mL amyloglucosidase in acetate buffer, dilute 2.3 mL amyloclucosidase (3260 U/mL) into 247.7 mL acetate buffer.

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**
>
> Leaving the lid off, especially if it is a shaking water bath, will help cool. For faster cooling, remove some water from the water bath and refill with cool distilled water. Do not use tap water as mineral buildup or heated chlorine could damage the water bath.

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. 30m

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.