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Total Starch Enzymatic Digestion

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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

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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

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

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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

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.

α-amylase comes at 1000 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.

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

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the volume of amyloglucosidase and acetate buffer to use.

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.

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.

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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 Place the tubes in ice to stop the reaction, until cool to the touch.

17 Proceed to total starch (as glucose) quantification by NZYtech GOD-POD method or store the samples at -20°C up to one month.

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