Dec 23, 2021

Total Starch (as Glucose) Quantification by NZYtech GOD-POD Method

DOI
dx.doi.org/10.17504/protocols.io.b27yqhpw

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Protocol Citation: Lynn Doran, Amanda P. De Souza 2021. Total Starch (as Glucose) Quantification by NZYtech GOD-POD Method. **protocols.io https://dx.doi.org/10.17504/protocols.io.b27yqhpw**

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

Created: December 22, 2021

Last Modified: July 11, 2023

Protocol Integer ID: 56280

Keywords: Starch, GOD-POD, Plant Tissue, Carbohydrates, Total Starch as Glucose

Funders Acknowledgements:
Realizing Increased Photosynthetic Efficiency (RIPE) that is funded by the Bill & Melinda Gates Foundation, Foundation for Food and Agriculture Research, and the U.K. Foreign, Commonwealth & Development Office
Grant ID: OPP1172157

Abstract

Total starch was previously extracted from plant tissue and enzymatically digested to D-Glucose. This protocol is based on the NZYtech GOD-POD kit's colorimetric determination of D-Glucose and includes basic calculations to convert D-Glucose back to total starch in the original tissue.

Guidelines

A standard curve is necessary for each day of measurement. If you are doing multiple plates in the same day with turning off the plate reader, it is okay have the standard curve only in one plate. For the standard curve, use the following amounts of glucose (Glc): 0, 2.5, 5, 7.5, 10, 12.5 ug. Include the 0 ug glucose/well on all plates.

Analyze samples in 3 technical replicates.

R2 of standard curve should be >0.995. Typical standard deviation between technical sample replicates is 0.00 and 0.06 depending on precision of multi-channel or repeat pipettor used.

This method is a modification of **NZYTech Standard Protocol** and **NZYTech Alternative Procedures**.

Materials

Reagents

- Glucose solution 1mg/mL, Sigma G6918-100 mL or Reagent 3 from Nzytech D-Glucose GOD-POD Kit Product AK00161.
- GOD-POD reagent, prepared per kit instructions, (Nzytech D-Glucose GOD-POD Kit Product AK00161)

**The GOD-POD reagent is enzymatic. If lower than expected absorbances are observed repeatedly or if reagent 2 seems clumpy when added to reagent 1, the enzyme may have gone bad. 100 ul of 1 mg/mL glucose with 3 mL of GOD-POD reagent should yield an ABS at 510 nm of 1-1.2.

Materials

- 96-well plates
- Pipette tips

Equipment

- Incubator, 40-50^{o}C
- Single channel pipette
- Multi-channel pipette
- UV-Vis Plate reader

Before start

Extract and dry total starch pellet from plant tissue per **Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues.** Enzymatically digest starch to glucose per **Total Starch Enzymatic Digestion**.

Prepare Glucose Standards

1 Prepare glucose standards in microcentrifuge by pipetting the appropriate amounts of 1 mg/mL Glucose standard and distilled water into each labeled tube.

	A	B	C
	A	B	C
	ug Glucose/20 ul-well (ug)	Amount 1 mg/mL Glucose (ul)	Amount distilled water (ul)
	0	0	80
	2.5	10	70
	5	20	60
	7.5	30	50
	10	40	40
	12.5	50	30

2 Pipette 20 ul of each prepared glucose standard in triplicate into the assigned wells.

Sample Preparation

3 Extract and digest total starch from plant tissue per **Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues.** and **Total Starch Enzymatic Digestion**.

4 Pipette 5-20 ul of each sample extract in triplicate into the assigned wells. Record the amount of sample added.

Note

The final absorbance of the sample must fall between the range of absorbances for the standard curve and ideally between 2.5-12.5 ug glucose standards. The volume of sample added to the 96 well plate will have to be adjusted depending on the amount of total starch in the sample.

For Maverick soybean leaf tissue 20 ul was sufficient for the majority of the samples with a few containing high enough starch to require sample volumes of either 5 ul or 10 ul.

Assay

5 Prepare GOD-POD reagent per the instructions in the NZYtech GOD-POD protocol. Follow shelf life and storage requirements as listed in the NZYtech GOD-POD protocol.

6 Add 300 uL of prepared GOD-POD in each well using the multi-channel.

Note

Quick and even distribution of GOD-POD reagent is critical for accurate and even color development between each sample well.

7 Incubate the plates at 45°C for 20 min.

Note

UIUC IGB Specific: If a 45°C incubator is not available in GEGC, dry oven 8 or 9 in CABBI theme can be reserved and set to desired setpoint. Reservations can be made through the **CABBI equipment booking portal.**

8 Read the absorbance at 510 nm on a UV-VIS spectrophotometer.

Additional Assay Plates

9 Do not shutoff the spectrophotometer lamp between plates. If the lamp remains on continuously, only one glucose standard curve is needed. If the lamp is shut off, a standard curve will need to be included.

10 Include a blank, 0 ug glucose standard (20 ul distilled water) on every plate.

Basic Calculations

11 Normalize each assay plates absorbances to zero using the 0 ug glucose standard per plate.

12 Calculate ug total starch as glucose for each sample using the averaged normalized standard curve absorbances for technical replicates and the averaged normalized

sample absorbances for technical replicates.

13 Divide the ug total starch by the ul of total sample extract loaded.

14 Multiply the ug total starch per ul of total sample extract loaded by the total number of uls of buffer the total starch was re-suspended in. If following the protocol, **Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues.** and **Total Starch Enzymatic Digestion**. , as written the total starch was resuspended in 2000 uls of buffer.

15 Divide the ug total starch per 2 mL extract by the initial weight of ground tissue used in the ethanolic extraction (Step 1 of **Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues.**) The final value reported will be ug Total Starch (as glucose) per mg plant tissue.