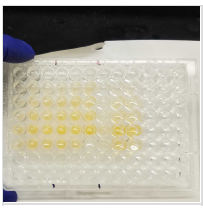

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Total Soluble Sugar Quantification from Ethanolic Plant Extracts

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

1

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Realizing Increased Photosynthetic Efficiency (RIPE)

Quantification of total soluble sugars (as glucose) in plant tissue extracts via the sulfuric phenol method adapted for 96 well plates.

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protocol

Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S, Lee YC. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal Biochem. 2005 Apr 1;339(1):69-72. doi: 10.1016/j.ab.2004.12.001. PMID: 15766712. https://pubmed.ncbi.nlm.nih.gov/15766712/

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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, 5, 10, 15,20, 25 ug. Include the 0 ug glucose/well on all plates.

Analyze samples in 3 technical replicates.

R^2 of standard curve should be >0.990. Typical standard deviation between technical sample replicates is 0.10 and 0.30 depending on precision of multi-channel or repeat pipettor used.

Reagents

- Sulfuric acid, Merck/Millipore brand, ACS grade
- Phenol 5% w/v

  For 100 mL: 5 g phenol up to 100 mL with milliQ water.

  Prepared fresh daily. ABS will start to fade at higher glucose levels the older the phenol gets.
- Glucose solution 1mg/mL to be used as a standard, Sigma G6918-100 mL Lot SLCD7032
- MilliQ or distilled water

Materials

- Pipette tips
- 96-well plates, polypropylene or PTFE only.

  Polystyrene is not chemical resistant and the sulfuric and phenol will interfere with plastic instead of glucose and cause high standard deviations and poor linearity of standard curves. Only use chemical resistant plastics.

Equipment

- Single channel pipette
- Multi-channel pipette
- Repeat pipettor
- Analytical Balance
- Water bath
- Ice bucket
- Chemical Fume Hood
- UV-Vis Plate reader

- This protocol uses chemical fume hoods. Understand how to safely and appropriately use a chemical fume hood performing the protocol.

- Phenol and sulfuric acid pose serious health risks and ethanol is flammable. Please read all manufacturer safety data sheets before handling. UIUC personnel performing this protocol should be current on "Laboratory Safety", "Chemical Safety- An Introduction", and "Chemical Spills" Division of Research Safety training

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modules before performing this protocol.

Extract and purify total soluble sugars from plant tissue per Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues.

Glucose standard preparation

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
ug Glucose/50 ul-well (ug)	Amount 1 mg/mL Glucose (ul)	Amount distilled water (ul)
5	20	180
10	40	160
15	60	140
20	80	120
25	100	100

2 Pipette 50 ul of each prepared glucose standard in triplicate into the assigned wells.

Sample preparation

3 Extract and purify total soluble sugars from plant tissue per Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues.

4 Pipette 10-30 ul of each sample extract in triplicate into the assigned wells. Record the amount of sample added.

The final absorbance of the sample must fall between the range of absorbances for the standard curve and ideally between 5-25 ug glucose standards. The volume of sample added to the 96 well plate will have to be adjusted depending on the amount of total soluble sugar in the sample.

For Maverick soybean leaf tissue 30 ul was used for Dawn and Dusk Sampling, 20 ul for mid-day sampling.

5 Add distilled water to bring the total volume of each well to 50 ul. For example, if 10 ul of sample was used add 40 ul of distilled water.

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Assay 5m

6 Move the plate to the chemical fume hood.

7 

Inside the hood, add 150uL of sulfuric acid to each well. Try to minimize the time between addition to first well and final well.

A multi-channel pipette or repeat pipettor will allow the fastest addition of sulfuric acid to all samples. The sulfuric acid will degrade the o-rings on the multi-channel pipette and reduce precision over repeated runs. If using a multi-channel, keep extra channel o-rings in stock.
Filter tips may help prolong the life of multi-channel pipette parts but they also decreased precision. Repeat pipettor is the best equipment for this protocol as the sulfuric is contained in disposable parts.

Sulfuric acid is highly corrosive. Wear proper PPE at all times. Keep in chemical fume hood at all times. Clean up any spills immediately.

8 

Immediately after the addition of sulfuric acid, add 30uL of phenol 5% in each well. Try to minimize the time between addition to first well and final well.

A multi-channel pipette or repeat pipettor will allow the fastest addition of phenol to all samples. The phenol will degrade the o-rings on the multi-channel pipette and reduce precision over repeated runs. If using a multi-channel, keep extra channel o-rings in stock. Filter tips may help prolong the life of multi-channel pipette parts but they also decreased precision. Repeat pipettor is the best equipment for this protocol as the sulfuric is contained in disposable parts.

This reaction produces heat, be careful. Phenol is toxic. Wear proper PPE at all

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times. Keep in chemical fume hood at all times. Clean up any spills immediately.

9 Incubate the plate by floating in a **90 °C** water bath for **00:05:00** . 5m

10 Place plate on ice bath to cool until cool to the touch.

11 Once the plate is cool, read the absorbance at 490 nm on a UV-VIS spectrophotometer.

Additional Assay Plates 5m

12 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.

13 Include a blank, 0 ug glucose standard (50 ul distilled water) on every plate.

Basic Calculations 5m

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

15 Calculate ug total soluble sugar as glucose for each sample using the averaged normalized standard curve absorbances for technical replicates and the averaged normalized sample absorbances for technical replicates.

16 Divide the ug total soluble sugar by the ul of total sample extract loaded.

17 Multiple the ug total soluble sugar per ul of total sample extract loaded by the total number of uls of distilled water the total soluble sugars were re-suspended in. If following the protocol, Extraction of Non-Structural Carbohydrates (Total Soluble Sugars + Starch) in Plant Tissues., as written the total soluble sugar was resuspended in 1000 uls of distilled water.

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18 Divide the ug total soluble sugar per 1 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 Soluble Sugars (as glucose) per mg plant tissue.

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