

Plant Soluble Sugar Assay Kit

Catalog No: SH0270

Method: Anthrone Colorimetric Method

Specification: 50T/48 samples

1. Reagent Composition and Preparation: (50T/48 samples)

Reagent 1: Powder ×1 bottle, store in the dark at 4°C;

Reagent 2: Liquid ×1 bottle, store sealed at 4°C;

Preparation of substrate solution: Add 5mL of Reagent 2 to the powder of Reagent 1, dissolve thoroughly. If it is difficult to dissolve, gently heat while stirring or shaking, and use once dissolved; the prepared substrate solution can be stored at 4°C in the dark for one week.

Reagent 3: 1mg/mL standard reserve solution 0.3mL ×1 tube, store at 4°C;

Standard diluent solution 10mL ×1 tube, store at 4°C;

Preparation of 100µg/mL standard application solution: Take 0.2mL of the 1mg/mL standard reserve solution and add it to 1.8mL of the standard diluent solution (i.e., standard reserve solution: standard diluent solution = 1:9), mix well to prepare the 100µg/mL standard application solution.

Reagent 4: Concentrated sulfuric acid (60mL-100mL) (analytical grade), self-provided.

2. Operating Steps:

- 1) **Extraction of soluble sugars:** Weigh 0.1 to 0.2g of the sample to be tested (fresh plant leaves, clean the surface dirt, chop and mix), add distilled water in a ratio of sample weight (g): distilled water (mL) = 1:10, grind into a homogenate using a homogenizer, pour into a capped centrifuge tube, and heat in a boiling water bath for 10 minutes (cap tightly, make a small hole in the tube cap to balance air pressure and reduce water loss), cool down, centrifuge at 4000 RPM at room temperature for 10 minutes, take the supernatant and dilute it with distilled water 10 times, mix well to prepare the sample supernatant for later use.

2) Operation Table:

Reagent	Blank Tube	Standard Tube	Test Tube
Distilled water (µL)	200		

100µg/mL standard application solution (µL)		200	
Sample supernatant (µL)			200
Substrate solution (µL)	100	100	100
Concentrated sulfuric acid (µL)	1000	1000	1000
Mix well, place in boiling water bath (95–100°C) to react for 10 min (during the water bath, the tube cap or sealing must be tight, with a small hole in the tube cap or sealing film to reduce water loss and balance air pressure), take out and cool in running water, wavelength 620nm, use a 1cm light path 4mm inner diameter (or 0.5cm light path) cuvette, zero with distilled water, measure the absorbance values of each tube ($\Delta A = A_{\text{Test tube}} - A_{\text{blank tube}}$).			

Note:

- Generally, only 1-2 blank tubes and standard tubes are needed.
- Before the formal experiment, a preliminary test must be done. If the ΔA value of the supernatant of the test sample ($\Delta A = A_{\text{Test tube}} - A_{\text{blank tube}}$) is greater than 1, the sample needs to be diluted with distilled water before measurement, and the corresponding dilution factor should be multiplied when calculating.
- As concentrated sulfuric acid is highly corrosive, please operate with caution; assays should use hard plastic centrifuge tubes or glass test tubes. If using glass test tubes, cover the mouth of the tube with plastic film, making a small hole in the film to maintain air pressure and reduce volatilization of the reaction solution.

3. Calculation Formulas:

1. Standard correction calculation method:

$$\text{Soluble Sugar } \mu\text{g/g FW} = \frac{\Delta A_{\text{Test}}}{\Delta A_{\text{Standard}}} \times C_{\text{Standard}} \div \frac{W}{V_{\text{extraction}}} \times N$$

Note: W is the fresh weight of the tissue, g; $V_{\text{extraction}}$ is the total volume of the extracting solution (distilled water), mL; N is the dilution factor before testing the sample.

2. Standard curve fitting calculation method:

(1) Insert ΔA into the regression equation obtained under standard conditions to determine the sugar content.

(2) Calculate based on the sample's fresh weight:

$$\text{Soluble Sugar} \frac{\mu\text{g/g FW}}{=} = \frac{\text{Sugar content (standard - based calculation)} \times V_{\text{sample}} \times N}{W}$$

Note: V_{sample} is the volume of the sample added, 0.2mL; W is the sample's fresh weight, g; N is the dilution factor before testing the sample.

4. Calculation Example:

Take 0.1g of plant leaf tissue, add distilled water in a ratio of sample weight (g): distilled water (mL) = 1:10, grind into a homogenate using a homogenizer, pour into a capped centrifuge tube, boil in a water bath for 10min (cap tightly, make a small hole in the tube cap to balance air pressure and reduce water loss), cool in running water, centrifuge at 4000 RPM for 10 minutes; simultaneously take part of the supernatant and dilute with distilled water 10 times, mix well to prepare the sample supernatant. Measurement data is as follows: blank tube OD is 0.039, standard tube OD is 0.530, Test tube OD is 0.651. Calculation results:

$$\text{Soluble Sugar} \frac{\mu\text{g/g FW}}{=} = \frac{(0.651 - 0.039)}{(0.531 - 0.039)} \times 100 \div \frac{0.1}{1} \times 10 = 12439.05 \mu\text{g/gFW}$$

5. Significance of Measurement:

Carbohydrates are one of the important components constituting plant bodies, and they are also the main raw materials and storage substances for metabolism. Different cultivation conditions and maturity can affect the sugar content in fruits and vegetables. Therefore, measuring soluble sugars in fruits and vegetables can help understand and identify the quality of fruits and vegetables. Total sugar refers to the reducing monosaccharides and sucrose, maltose, and starch that can be hydrolyzed into reducing monosaccharides under the conditions of this method.

6. Measurement Principle:

Sugars, under the action of concentrated sulfuric acid, can undergo dehydration reactions to produce sugar aldehydes or hydroxymethyl sugar aldehydes. The resulting sugar aldehydes or hydroxymethyl sugar aldehydes can react with anthrone to produce blue-green furfural derivatives. Within a certain range, the depth of color is proportional to the sugar content, thus allowing for quantitative analysis of sugars. The colored substances generated from the reaction of carbohydrates with anthrone show an absorption peak in the visible region at 630nm, where colorimetry can be performed. The anthrone colorimetric method used in this kit can be employed to measure the content of soluble monosaccharides, oligosaccharides, and polysaccharides, boasting high sensitivity, simplicity, speed, and suitability for measuring trace samples.

Annex I: Standard Curve of Soluble Sugars

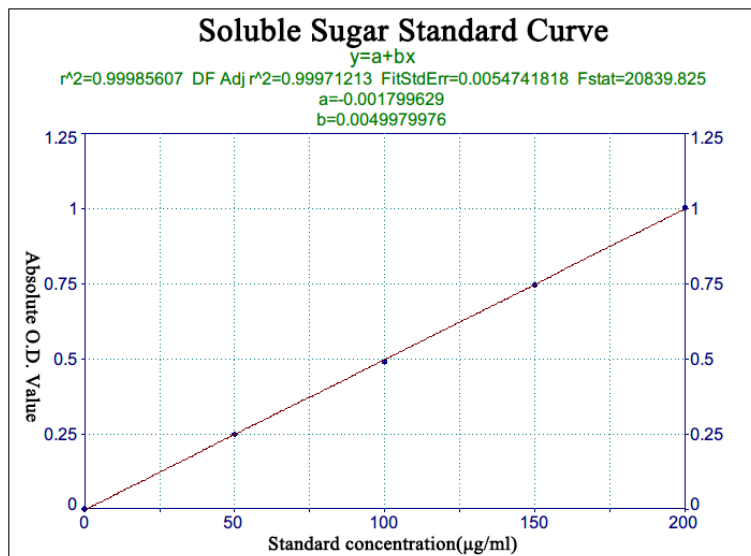
1. Preparation of the Standard Curve

Dilution Tube	S1	S2	S3	S4	S5
200µg/mL Standard Application Solution	0µL	50µL	100µL	150µL	200µL
Double Distilled Water	200µL	150µL	100µL	50µL	0µL
Substrate Solution	100µL	100µL	100µL	100µL	100µL
Concentrated Sulfuric Acid	1000µL	1000µL	1000µL	1000µL	1000µL
Mix, place in boiling water bath (95–100 °C) for 10min (cover tightly, make a small hole in the tube cap to reduce water loss), cool in running water, 620nm wavelength, use a 1cm light path cuvette, measure the absorbance values after zeroing with distilled water.					
Equivalent Sugar Content	0µg/mL	50µg/mL	100µg/mL	150µg/mL	200µg/mL

2. Measurement Results (with reference standard curve):

Standard Tube	Equivalent Sugar Content (µg/mL)	Measurement OD	Absolute OD
S1	0	0.032	0.000
S2	50	0.283	0.251
S3	100	0.524	0.492
S4	150	0.776	0.744
S5	200	1.035	1.003

Using absolute absorbance as the vertical coordinate and sugar content as the horizontal coordinate, plot the standard curve and derive the standard linear equation. The regression equation obtained under standard conditions is $y = a + bx$, where x is the concentration of the standard solution ($\mu\text{g/mL}$) and y is the absolute absorbance.



[Note]: For ease of calculation, using absolute absorbance as the horizontal coordinate and sugar content as the vertical coordinate, derive the standard linear equation. The regression equation obtained under standard conditions is $y = a + bx$, where X is absolute absorbance and y is the concentration of the standard solution ($\mu\text{g/mL}$).

