Plant soluble sugar content test kit

(Cat.No:BC095 Size:50T/48S)

1. Composition and preparation (The kit is valid for 3 months)

Reagent 1: powder x1 bottle, 4℃ store in the dark;

Reagent 2: liquid x 1 bottle, 4℃ sealed for storage;

Preparation of substrate solution: Add 5mL reagent II to the reagent I powder agent and dissolve it thoroughly. If it is difficult to dissolve, stir or shake slightly with a slight heat. After dissolution, it can be used; the prepared substrate solution 4℃ can be stored for one week under light.

Reagent 3: 1mg/mL standard storage solution 0.3mL x 1, 4℃ for storage;

Standard dilution solution 10mL x 1 vial, 4℃ for storage;

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

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

2. Operation steps

1. **Extraction of Soluble Sugars:** Weigh the sample to be tested 0.1~0.2g (fresh plant leaves, dry surface contaminants, chop and mix), add distilled water at a ratio of sample weight (g): distilled water (mL) = 1:10, blend with a homogenizer, pour into a capped centrifuge tube, incubate in boiling water bath for 10min (cover tightly, puncture the cap slightly to balance pressure and minimize water loss), cool, then centrifuge at 4000 rpm for 10min at room temperature. Take the supernatant, dilute 10 times with distilled water, shake well to prepare the sample supernatant for later use. **(For protein concentration correction, additional protein quantification reagent kits need to be ordered; these are available in our laboratory.)**
2. **Operation table:**

reagent	Blank tubes	Standard Tube	Determination tube
distilled water (μL)	200		
100μg/mL standard application solution (μL)		200	
Sample supernatant (μL)			200
Bath solution (μL)	100	100	100
concentrated sulfuric acid (μL)	1000	1000	1000

Stir thoroughly and place in a boiling water bath (95-100°℃) for 10 minutes (cover the tube tightly or seal it during water bath, and puncture a small hole in the cap or sealing membrane to minimize water loss and pressure equilibrium). After cooling with running water, measure at 620nm using a cuvette with a 1cm path length and an inner diameter of 4mm (or 0.5cm path length), adjust to zero with distilled water, and determine the absorbance values of each tube ($\Delta A = A_{measured\ tube} - A_{blank\ tube}$).

Note: 1. Generally, only 1-2 tubes of blank tube and standard tube are made; 1cm light

diameter and 4mm inner diameter cuvette or 0.5cm light

diameter cuvette are available for sale.

2. A preliminary experiment should be conducted before the formal test. If the ΔA value

(ΔA=A measurement tube-A blank tube) of the supernatant of the

sample to be tested is greater than 1, the sample should be diluted

with distilled water before

measurement, and the corresponding dilution factor should be multiplied in

calculation.

3. Due to the strong corrosiveness of concentrated sulfuric acid, please operate with

caution; hard plastic centrifuge tubes or glass test tubes are used for measurement. If glass test tubes are used, the test tube mouth can be sealed with a plastic film, and a

small hole can be punched on the film to maintain the air pressure and reduce the volatilization of the reaction liquid.

3. Calculation formula

1. Standard correction calculation method:

$$\text{Soluble sugar content (μg/gWet weight)} = \frac{\Delta A_{\text{Determination}}}{\Delta A_{\text{standards}}} \times C_{\text{standards}} \div \frac{W}{V_{ext}} \times N$$

Note: W is fresh weight of the tissue, g; V_{ext} is the total volume of the extract (distilled water) added, mL; N is the dilution factor before sample testing.

2. Standard curve fitting calculation method:

(1)Substitute ΔA into the regression equation measured under standard conditions to obtain the sugar content.

(2)Calculated according to fresh weight of samples:

$$\text{Soluble sugar content (μg/gWet weight)} = \frac{\text{Calculate sugar content from the standard curve} \times V_{sample} \times N}{W}$$

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

4. Calculation example

Take 0. 1g of plant leaf tissue and add distilled water at a ratio of sample mass (g): distilled water (mL) = 1:10. Homogenize the mixture with a homogenizer, then transfer it to a capped centrifuge tube. Incubate in boiling water for 10 minutes (cover tightly, puncture the cap slightly to balance pressure and minimize water loss). After cooling, centrifuge at 4000 rpm for 10 minutes at room temperature. Simultaneously, take part of the supernatant and dilute it 10 times with distilled water, shake well to prepare the sample supernatant. The measured data are as follows: OD of the blank tube is 0.039, OD of the standard tube is 0.530, and OD of the test tube is 0.651. Calculation results:

$$\text{Soluble sugar content (μg/gWet weight)} = \frac{(0.651-0.039)}{(0.531-0.039)} \times 100 \div \frac{0.1}{1} \times 10$$

= 12439.05μ2/gWet weight = 12.439mg/gWet weight

5. Measurement significance

Carbohydrates are one of the essential components that make up plant bodies and serve as the primary raw material for metabolism and storage substances. Different cultivation conditions and varying maturity stages can affect the sugar content in fruits and vegetables. Therefore,

measuring soluble sugars in fruits and vegetables can help understand and evaluate their quality. Total sugar refers to the reducing monosaccharides in the sample, as well as sucrose, maltose,

and starch that can partially hydrolyze into glucose under the conditions of this method.

6. Determination principle

Sugar (sugar) can undergo dehydration reactions under the action of concentrated sulfuric

acid to form aldose or hydroxymethylaldose. The generated aldose or hydroxymethylaldose can react with anthrone to produce blue-green furfural derivatives. Within a certain range, the depth of color is proportional to the amount of sugar, making it suitable for quantitative determination of sugar. The colored substances formed by the reaction between sugars and

anthrone have an absorption peak at 630nm in the visible light region, allowing for colorimetric analysis at this wavelength. This kit uses the anthrone colorimetric method, which is applicable for the determination of soluble monosaccharides, oligosaccharides, and polysaccharides, offering advantages such as high sensitivity, simplicity, and rapidity, as well as suitability for trace sample analysis.

Appendix I: Standard curve of soluble sugar

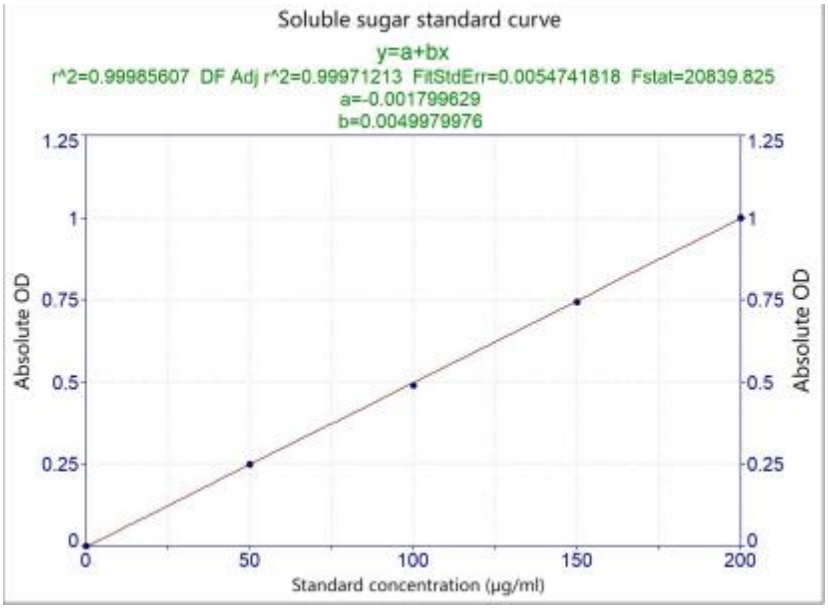
I. Preparation of standard curve

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 and place in boiling water bath (95~100℃) for 10min (cover tightly and make a small hole on the tube cap to reduce water loss), cool with running water, measure the absorbance value of each tube at 620nm wavelength and 1cm optical path ratio after adjusting the zero point with distilled water.					
It's the same as sugar content	0μg/mL	50μg/mL	100μg/mL	150μg/mL	200μg/mL

2. Test results (with reference standard curve attached):

Standard quality control	Equivalent to sugar content (μg/mL)	determine 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

The standard curve was drawn with the absolute absorbance value as the vertical coordinate and the sugar content as the horizontal coordinate, and the standard linear equation was obtained. The regression equation of the measurement under the standard condition was y=a+bx, where x was the concentration of the standard (μg/mL) and y was the absolute absorbance value.



[Note]: For convenience of calculation, the absolute absorbance value is taken as the horizontal coordinate and the sugar content as the vertical coordinate, and the standard linear equation is obtained. The regression equation measured under standard conditions is y=a+bx, where X is the absolute absorbance value and y is the concentration of the standard (μg/mL).