Plant Soluble Sugar Colorimetric Assay Kit

Cat #: orb1173209 (manual)

Product name: Plant Soluble Sugar Colorimetric Assay Kit

Catalog number: orb1173209

Detection range: 0.0125-0.5 mg/mL

Sensitivity: 0.0125 mg/mL

Applicable samples: Plant

Storage: Stored at 4℃ for 6 months, protected from light

Assay Principle

Carbohydrates are one of the important components of plant bodies, and are also the main raw materials and storage materials for metabolism. Soluble sugars refer to the reducing monosaccharides in the sample, sucrose and maltose that can be hydrolyzed into reducing monosaccharides under the determination conditions of this method, and starch that can be partially hydrolyzed into glucose. The detection principle is anthrone colorimetry. It can be used for the determination of soluble monosaccharides, oligosaccharides and polysaccharides. It has the advantages of high sensitivity, simplicity and speed, and suitable for the determination of trace samples.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Anthrone	Powder×1 vial	4℃, protected from light
Solvent	10 mL	4℃
Glucose Standard	Powder×1 vial (10 mg)	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 620 nm
- 96-well plate or microglass cuvette
- Ice maker, centrifuge
- Incubator or metal bath
- Precision pipettes, disposable pipette tips
- Deionized water
- Concentrated sulfuric acid
- Homogenizer

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Reagent Preparation

Working Solution: Add 3 mL Solvent into Anthrone, and fully dissolve it for later use. If it is difficult to dissolve, it can be heated and stirred. The remaining working solution can be stored at 4℃ for one week.

Note: Solvent is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Glucose Standard: Before use, add 1 mL deionized water to the 10 mg Glucose Standard to prepare 10 mg/mL standard substance, which could be stored at 4℃ for one week.

Setting of Standard curve: Further dilute the standard to 0.5, 0.2, 0.1, 0.05, 0.025, 0.0125 mg/mL standard with deionized water, as shown in the following table:

Num.	Volume of Glucose Standard	Volume of Deionized Water	Concentration
Std.1	50 µL 10 mg/mL	950 µL	0.5 mg/mL
Std.2	80 µL of Std.1 (0.5 mg/mL)	120 µL	0.2 mg/mL
Std.3	100 µL of Std.2 (0.2 mg/mL)	100 µL	0.1 mg/mL
Std.4	100 µL of Std.3 (0.1 mg/mL)	100 µL	0.05 mg/mL
Std.5	100 µL of Std.4 (0.05 mg/mL)	100 µL	0.025 mg/mL
Std.6	100 µL of Std.5 (0.025 mg/mL)	100 µL	0.0125 mg/mL

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Plant: Weigh about 0.1 g sample, add 1 mL deionized water to homogenize it or grind it into homogenate, pour it into a centrifuge tube with a cap, take incubator or metal bath at 95℃ for 10 min (cover tightly to prevent water loss), after cooling, Centrifuge at 8,000 g for 10 min at 25℃, take the supernatant into 10 mL test-tube, volume it to 10 mL with deionized water, and shake well for standby.

Assay Procedure

1. Preheat the microplate reader or Visible spectrophotometer for more than 30 min, and adjust the wavelength to 620 nm, Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement (the following operations are operated in the EP tube):

Reagent	Blank Tube (µL)	Test Tube (µL)	Standard Tube (µL)
Sample	0	40	0
Standard	0	0	40
Deionized Water	80	40	40
Working Solution	20	20	20
Concentrated sulfuric acid	200	200	200

3. Mix well, incubate in 95°C water bath or metal bath for 10 min (cover tightly to prevent water loss),

cool to room temperature, take 200 µL into 96-well plate or microglass cuvette, and read the absorbance values of the blank tube, test tube and standard tube at 620 nm, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Only one blank tube needs to be made. If the ΔA_{Test} values are higher than 1.6, dilute sample with deionized water and repeat this assay, multiply the results with the dilution factor. Concentrated sulfuric acid is highly corrosive, please handle it carefully.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculating the content of soluble sugar:

According to the standard curve, the ΔA_{Test} is substituted into the formula (x) to calculate the sample concentration y (mg/mL).

(1) Calculated by protein concentration

soluble sugar (mg/mg prot)=$(y\times V_1)\div(V_1\times Cpr)$**=y÷Cpr**

(2) Calculated by fresh weight of samples

soluble sugar (mg/g fresh weigh)=$(y\times V_1)\div(W\times V_1\div V_2)$**=10×y÷W**

Where: V_1: sample volume added, 0.04 mL; Cpr: sample protein concentration, mg/mL; W: fresh weigh of samples, g; V_2: the volume of total samples, 10 mL.

Typical Data

Typical standard curve:

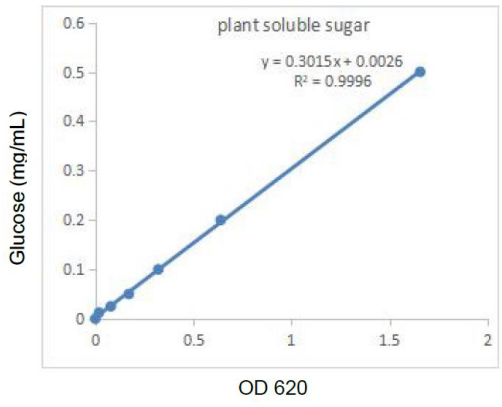

Figure 1. Standard curve of Glucose Standard assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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