



TECHNICAL MANUAL

Plant Soluble Sugar Colorimetric Assay Kit

- **SKU CODE:** MAES0270
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Reagent preparation
- Sample preparation
- Add reagents to blank, standard, and sample tubes
- Heat incubation and cooling
- Measure absorbance at 620 nm

2. Intended use

This kit can measure soluble sugar content in plant samples.

3. Detection principle

Carbohydrates are important components of plant tissues and serve as the main raw materials for metabolism and energy storage. This kit is designed for the determination of soluble monosaccharides, oligosaccharides, and polysaccharides. It offers high sensitivity, convenience, and is suitable for microscale sample analysis.

The detection principle is based on anthrone colorimetry. Carbohydrates react with anthrone to produce colored compounds, which exhibit maximum absorption at 620 nm. The soluble sugar content is determined by measuring the absorbance value.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Substrate	Powder x 1 vial	Powder x 2 vials	2-8 °C, 12 months, protected from light
Reagent 2	1 mg/mL Standard	1 mL x 1 vial	1 mL x 2 vials	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (610-630 nm, optimal wavelength: 620 nm)

Reagents:

Concentrated sulfuric acid, Ethyl acetate

6. Reagent preparation

1. Allow all reagents to equilibrate to room temperature before use.
2. Preparation of substrate working solution: Dissolve one vial of substrate with 6 mL of ethyl acetate in a 60 °C water bath for 1-2 minutes until clear. Store at 2-8 °C for up to 7 days, protected from light.
3. Preparation of 0.1 mg/mL standard: For each well, prepare 200 µL of 0.1 mg/mL standard by mixing 20 µL of 1 mg/mL standard with 180 µL of double distilled water. Mix well. Store at 2-8 °C and use within 3 days.

7. Sample preparation

Sample preparation: Tissue sample: 1. Harvest the amount of tissue needed for each assay (initial recommendation: 30 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 30 mg tissue in 270 µL double distilled water with a dounce homogenizer at 4 °C. 4. Centrifuge at 10,000 x g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep on ice for detection.

Dilution of sample: The recommended dilution factors for different samples are as follows (for reference only):

10% Mango tissue homogenate: 150-250

10% Fresh jujube tissue homogenate: 150-250

10% Grape tissue homogenate: 150-200

10% Corn tissue homogenate: 100-200

10% Apple tissue homogenate: 100-150

10% Banana tissue homogenate: 150-200

10% Cucumber tissue homogenate: 20-50

10% Tomato tissue homogenate: 30-60

10% Mushroom tissue homogenate: 1

10% Carrot tissue homogenate: 40-60

Note: The diluent is double distilled water. For other sample types, perform a pretest to confirm the appropriate dilution factor.

8. The key points of the assay

1. The substrate working solution preparation should be incubated in a 60 °C water bath for 1-2 minutes until clear.
2. The heat release during the addition of concentrated sulfuric acid may cause liquid splashing. Add samples slowly along the tube wall.
3. It is recommended to add samples in a fume hood whenever possible.
4. The concentration of sulfuric acid must be over 95%. Concentrated sulfuric acid may absorb water during long-term storage, which may interfere with results.

9. Operating steps

1. Blank tube: Add 0.2 mL of double distilled water to a 2 mL EP tube. Standard tube: Add 0.2 mL of 0.1 mg/mL standard to a 2 mL EP tube. Sample tube: Add 0.2 mL of sample to a 2 mL EP tube.
2. Add 0.1 mL of substrate working solution and 1 mL of concentrated sulfuric acid to each tube.
3. Mix thoroughly and incubate the tubes at 95-100 °C for 7 minutes (secure the tube caps during heating), then cool the tubes immediately with running water.
4. Transfer 200 µL of the reaction solution from each tube to the microplate using a micropipette. Measure the OD value of each well at 620 nm using a microplate reader.

10. Calculation

The sample:

$$\text{Soluble sugar content (mg/g wet weight)} = (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{standard}} - A_{\text{blank}}) \times C_{\text{standard}} / W \times V \times f$$

[Note]

A_{standard} : The OD value of standard well.

A_{blank} : The OD value of blank well.

A_{sample} : The OD value of sample well.

C_{standard} : The concentration of standard, 0.1 mg/mL.

W: The weight of the sample, g.

V: The volume of homogenate, mL.

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three banana tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.09	0.53	1.20
%CV	2.2	1.7	1.2

Inter-assay Precision

Three banana tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.09	0.53	1.20
%CV	5.1	4.9	5.9

Recovery

Three samples of high, middle, and low concentrations were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 102%.

Sample	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	0.06	1.05	1.4
Observed Conc. (mg/mL)	0.1	1.1	1.4
Recovery rate (%)	103	102	101

Sensitivity

The analytical sensitivity of the assay is 0.003 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

12. Appendix II Example Analysis

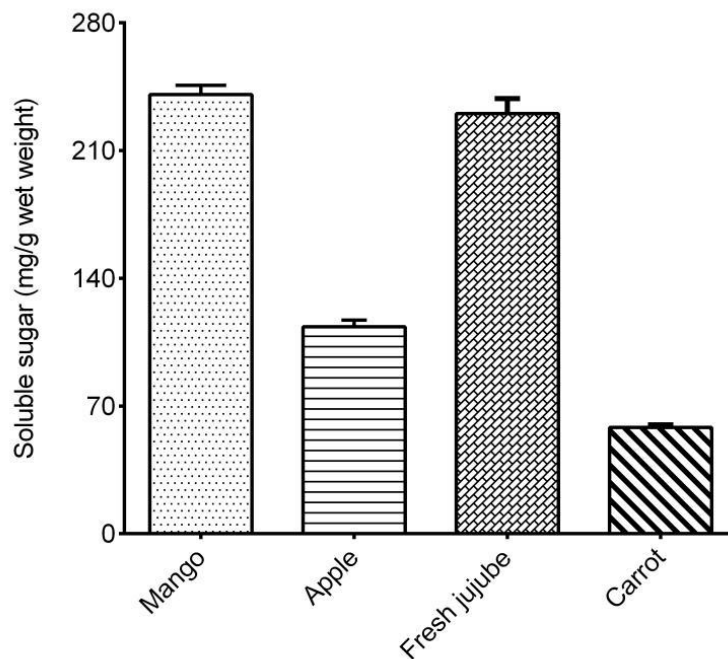
Example analysis:

For 10% mango tissue homogenate, dilute 200-fold, then take 200 μ L sample and perform the assay according to the operating steps. The results are as follows:

The value of the blank well is 0.068, the value of the standard well is 0.375, the value of the sample well is 0.478, and the calculation result is:

$$\text{Soluble sugar content (mg/g wet weight)} = (0.478 - 0.068) \div (0.375 - 0.068) \times 0.1 \div (0.1 \div 0.9) \times 200 = 240.39 \text{ mg/g wet weight}$$

Detect 10% mango tissue homogenate (diluted 200-fold), 10% apple tissue homogenate (diluted 100-fold), 10% fresh jujube tissue homogenate (diluted 200-fold), and 10% carrot tissue homogenate (diluted 50-fold) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.