Technical Manual

Sucrose Colorimetric Assay Kit

- Catalogue Code: MAES0121
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.32-70 µmol/mL

Sensitivity:

0.32 µmol/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Sucrose is a disaccharide which composed by glucose and fructose and it is the main nutrient in most plant cells. The biosynthesis of sucrose depends on the catalysis of sucrose phosphate synthase and 6'-sucrose phosphate phosphatase. It can participate in glycolysis and tricarboxylic acid cycle to produce ATP and NADH.

3. Intended Use

This kit can be used to measure sucrose content in plant tissue samples.

4. Detection Principle

Sucrose in plant tissue is hydrolyzed to glucose and fructose in boiling water bath under acidic conditions. 5-hydroxymethyl furfural was synthesized from fructose under acid condition and measure the ultraviolet absorption of 5-hydroxymethyl furfural. Glucose must be dissimilated into ketose structure and reduced to obtain 5-hydroxymethylfurfural, but the rate of isomerization of glucose to ketose is very slow. Therefore, the ultraviolet absorption of glucose is much smaller than fructose.

5. Kit components & storage

Item	Specification	Storage
Hydrolysate Solution	60 mL × 2 vials	2-8°C, 6 months
Sucrose Standard (100 μmol/mL)	1 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (290 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. The temperature of the water bath must be stable above 95°C.

2. Glass tubes must be used in this experiment.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **sucrose standard (20 μmol/mL)**: Dilute sucrose standard (100 μmol/mL) with double distilled water at a ratio of 1:4. Prepare the fresh solution before use. Prepared solution can be stored at 2-8°C for 7 days.

8. Sample Preparation

Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.32-70 μmol/mL).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
10% Green pepper tissue homogenization	1
10% Epipremnum aureum tissue homogenization	1
10% Cucumber tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 290 nm

10. Operation Steps

1. **Blank tube:** add 0.03 mL of double distilled water into a 5 mL glass tube.
   Standard tube: add 0.03 mL of 20 μmol/mL sucrose standard into a 5 mL glass tube.
   Sample tube: add 0.03 mL of sample into a 5 mL glass tube.

2. Add 2.0 mL of hydrolysate solution and mix fully with a vortex mixer.

3. Tighten the tubes with preservative film and make a hole on the film. Incubate the tubes in 100°C water bath for 8 min. Cool the tubes with running water.

4. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 290 nm with 1 cm optical path quartz cuvette.

Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.03		
20 μmol/mL sucrose standard (mL)		0.03	
Sample (mL)			0.03
Hydrolysate Solution (mL)	2	2	2
Mix fully with a vortex mixer. Tighten the tubes with preservative film and make a hole on the film. Incubate the tubes in 100℃ water bath for 8 min. Cool the tubes with running water. Set the spectrophotometer to zero with double-distilled water and measure the OD values of each tube at 290 nm with 1 cm optical path quartz cuvette.			

11. Calculations

$$\text{Sucrose concentration (μmol/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

c: The concentration of standard, 20 μmol/mL

f: Dilution factor of sample before tested

C_{pr}: Concentration of protein in sample, mgprot/mL

Email: info@assaygenie.com **Web:** www.assaygenie.com

12. Performance Characteristics

Detection Range	0.32-70 μmol/mL
Sensitivity	0.32 μmol/mL
Average recovery rate (%)	102
Average inter-assay CV (%)	8.5
Average intra-assay CV (%)	3.4

Analysis

Take 0.03 mL of 10% green pepper tissue homogenate, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.229, the average OD value of the blank is 0.001, the average OD value of the standard is 0.675, the concentration of protein in sample is 1.73 mgprot/mL, and the calculation result is:

$$\text{Sucrose concentration (μmol/mgprot)} = \frac{0.229-0.001}{0.675-0.001} \times 20 \div 1.73 = 3.91 \text{ (μmol/gprot)}$$

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory’s biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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