# Technical Manual

# Sucrose Fluorometric Assay Kit

- **Catalogue Code: MAES0013**
- **Size: 96T**
- **Research Use Only**

# 1. Key features and Sample Types

## Detection method:

Fluorimetric method

## Specification:

96T

## Range:

0.15-15 μmol/L

## Sensitivity:

0.15 μmol/L

## Storage:

-20°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, a kind of disaccharide, is a major component of sugar. It is widely found in the leaves, flowers, stems, seeds and fruits of plants. It is an important product of plant photosynthesis and a major form of storage, accumulation and transportation of sugar in plants. Sucrose biosynthesis is catalyzed by sucrose phosphate synthase and 6 '-sucrose phosphate phosphatase and can through glycolysis and tricarboxylic acid cycle to produce ATP and NADH. Sucrose is particularly rich in sugarcane, sugar beet and other sugar crops and fruits. It is an important condiment of food and sweet.

## 3. Intended Use

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

## 4. Detection Principle

Sucrose can be hydrolyzed by sucrase to produce glucose under acidic conditions, which is catalyzed by glucose oxidase to produce hydrogen peroxide. In the presence of HRP (horse radish peroxidase), hydrogen peroxide reacts with the fluorescent probe to form red fluorescent substance. The sucrose content can be calculated by measuring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

## 5. Kit components & storage

| Item | Specification | Storage |
|---|---|---|
| **Extraction Solution** | 45 mL × 1 vial | -20°C, 6 months |
| **Enzyme Reagent 1** | Powder × 1 vial | -20°C, 6 months, shading light |
| **Buffer Solution** | 10 mL × 1 vial | -20°C, 6 months |
| **Enzyme Reagent 2** | Powder × 1 vial | -20°C, 6 months, shading light |
| **Probe** | 0.25 mL × 1 vial | -20°C, 6 months, shading light |
| **Standard** | Powder × 1 vial | -20°C, 6 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/590 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

## 6. Assay Notes:

1. The volume of enzyme reagent 1 must be strictly controlled.

2. Fluorescent probe reaction must avoid direct sunlight.

## 7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. Preparation of **extraction working solution:** Mix the extraction solution and double distilled water at a ratio of 1:9 fully. Prepare the fresh solution before use. The prepared solution can be stored at 2-8°C for 7 days.

3. Preparation of **enzyme working solution 1:** Dissolve a vial of enzyme reagent 1 with 300 µL of double distilled water and mix fully. The prepared solution can be stored at -20°C for 7 days with shading light.

4. Preparation of **enzyme working solution 2:** Dissolve a vial of enzyme reagent 2 with 0.25 mL of double distilled water and mix fully. The prepared solution can be stored at -20°C for 7 days with shading light.

5. Preparation of **reaction working solution:** Mix the buffer solution, enzyme working solution 2 and probe at a ratio of 46:2:2 fully. Prepare the fresh solution before use.

6. Preparation of **standard (10 mmol/L):** Dissolve a vial of standard with 10 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 7 days. The preparation of **standard (100 µmol/L):** Dilute 10 µL of 10 mmol/L standard fully with 990 µL of reagent 1 working solution at a ratio of 1:99. Prepare the fresh solution before use.

## 8. Sample Preparation

**Plant tissue sample:**
Weigh o1 g tissue accurately. Add 0.9 mL of reagent 1 working solution in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 12000 g for 10 min at 4℃ and collect the supernatant for measurement. If not detected on the same day, the supernatant can be stored at -20°C for 5 days.

### 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 (0.15-15 μmol/L).

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

| Sample Type: | Dilution Factor |
|---|---|
| 10% Corn tissue homogenate | 1500 |
| 10% Potato tissue homogenate | 300-500 |
| 10% Tomato tissue homogenate | 200-300 |
| 10% Macrophanerophytes leaf tissue homogenate | 200-400 |
| 10% Carrot tissue homogenate | 1500 |
| 10% Onion tissue homogenate | 500-1000 |
| 10% Green pepper tissue homogenate | 500-1000 |
| 10% Bush leaves tissue homogenate | 20-50 |

**Note:** The diluent is extraction working solution.

## 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** Ex/Em=535 nm/590 nm

### Plate Set Up:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | A | A | S1 | S1’ | S9 | S9’ | S17 | S17’ | S25 | S25’ | S33 | S33’ |
| **B** | B | B | S2 | S2’ | S10 | S10’ | S18 | S18’ | S26 | S26’ | S34 | S34’ |
| **C** | C | C | S3 | S3’ | S11 | S11’ | S19 | S19’ | S27 | S27’ | S35 | S35’ |
| **D** | D | D | S4 | S4’ | S12 | S12’ | S20 | S20’ | S28 | S28’ | S36 | S36’ |
| **E** | E | E | S5 | S5’ | S13 | S13’ | S21 | S21’ | S29 | S29’ | S37 | S37’ |
| **F** | F | F | S6 | S6’ | S14 | S14’ | S22 | S22’ | S30 | S30’ | S38 | S38’ |
| **G** | G | G | S7 | S7’ | S15 | S15’ | S23 | S23’ | S31 | S31’ | S39 | S39’ |
| **H** | H | H | S8 | S8’ | S16 | S16’ | S24 | S24’ | S32 | S32’ | X | X |

**Note:** A-H, standard wells; X, blank wells; S1-S39, sample wells; S1’-S39’, control wells.

# 10. Operation Steps

## The preparation of standard curve

Dilute glucose standard (100 μmo/L) with extraction working solution to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 5, 8, 10, 12, 15 μmol/L.

## The measurement of samples

1. **Standard well:** Add 2.5 μL of enzyme working solution 1 into the corresponding wells.
   **Sample well:** Add 2.5 μL of enzyme working solution 1 into the corresponding wells.
   **Control well:** Add 2.5 μL of extraction working solution into the corresponding wells
   **Blank well:** Add 2.5 μL of extraction working solution into the corresponding wells.

2. Add 50 μL of standards with different concentrations into the standard wells.

3. Add 50 μL of sample into the sample and control wells.

4. Add 50 μL of extraction working solution into the blank wells.

5. Mix fully with microplate reader for 10 s and incubate at 37°C for 15 min.

6. Add 50 μL of reaction working solution into each well.

7. Mix fully with microplate reader for 10 s and incubate at 37°C for 30 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

## Operation Table

| | Standard well | Sample well | Control well | Blank well |
|---|---|---|---|---|
| **Enzyme working solution** 1 **(μL)** | 2.5 | 2.5 | | |
| **Extraction working solution (μL)** | | | 2.5 | 2.5 |
| **Standards with different concentrations (μL)** | 50 | | | |
| **Sample (μL)** | | 50 | 50 | |
| **Extraction working solution (μL)** | | | | 50 |
| Mix fully with microplate reader for 10 s and incubate at 37°C for 15 min. | | | | |
| **Reaction working solution (μL)** | 50 | 50 | 50 | 50 |
| Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm. | | | | |

# 11. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b

$$\text{Sucrose content (μmol/g wet weight)} = (\Delta F - b) \div a \times V \times f \div W \div 1000$$

**y:** $F_{Standard} - F_0$. ($F_0$ is the fluorescence value when the standard concentration is 0)
**x:** The concentration of standard
**a:** The slope of standard curve
**b:** The intercept of standard curve
**ΔF:** Absolute fluorescence intensity of sample ($\triangle F = (F - F_0) - (F' - F_0')$)
**F:** The fluorescence intensity of sample well
**F':** The fluorescence intensity of control well
**$F_0$':** The fluorescence intensity of blamk well
**V:** The total volume of tissue extraction, 0.9 mL
**f:** Dilution factor of sample before test
**W:** The weight of plant tissue, 0.1 g
**1000:** The coefficient of unit conversion

# 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 0.15-15 μmol/L |
| **Sensitivity** | 0.15 μmol/L |
| **Average recovery rate (%)** | 96 |
| **Average inter-assay CV (%)** | 6.5 |
| **Average intra-assay CV (%)** | 2.3 |

## Analysis

For 10% corn grain tissue homogenate, take 2.5 μL of sample supernatant diluted for 1500 times, carry the assay according to the operation table.

**The results are as follows:**

y = 1044.1 x + 12.953, the average of F is 10950, the average of F' is 1215, the average of $F_0$ is 3075, the average of $F_0$' is 957, ΔF = (10950 - 3075) – (1215 – 957) = 7617, and the calculation result is:

**Sucrose content (μmol/g wet weight)**
**= (7617-12.953) ÷ 1044.1 × 0.9 × 1500 ÷ 0.1 ÷ 1000**
**=98.32 μmo/g wet weight**



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## Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid 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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