TECHNICAL MANUAL

Sucrose Synthase-I Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0514
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Standard curve preparation
- Add standards and samples
- Incubation and chromogenic reaction
- Measure absorbance values

2. Intended use

This kit can be used to measure sucrose synthase-I (SS-I) activity in plant tissue samples.

3. Detection principle

Sucrose synthase-I (SS-I) catalyzes the substrate reaction to generate reducing sugar. The reducing sugar produces color changes when reacting with the chromogenic agent, showing a characteristic absorption peak at 540 nm. The activity of the enzyme is directly proportional to the absorbance.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	55 mL × 1 vial	-20°C, 12 months
Reagent 2	Buffer Solution	10 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate	Powder × 2 vials	-20°C, 12 months
Reagent 4	Chromogenic Agent	11 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 5	Standard	Powder × 2 vials	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (530-550 nm, optimum wavelength: 540 nm), Incubator, Water bath

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 2 mL of buffer solution, mix well. Store in aliquots at -20°C for up to one week.
3. Before using chromogenic agent, incubate in a water bath at 60-70°C for 10 minutes.
4. **Preparation of 2 mg/mL standard solution:** Dissolve one vial of standard with 5 mL of extraction solution, mix well. Store in aliquots at -20°C for up to one week.
5. **Preparation of standard curve:** Always prepare fresh standards. Discard working standard dilutions after use. Dilute 2 mg/mL standard solution with extraction solution to create a serial concentration series. The recommended dilution gradient is: 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 mg/mL.

7. Sample preparation

Sample preparation:

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Homogenize 20 mg tissue in 180 µL extraction solution with a dounce homogenizer at 4°C.

3. Centrifuge at 10,000 × g for 10 minutes at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection.

4. Meanwhile, determine the protein concentration of supernatant.

Dilution of sample:

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

- 10% Potato tissue homogenate: 1

- 10% Green peppers tissue homogenate: 1

- 10% Chinese cabbage leaf tissue homogenate: 1

- 10% Corn seed tissue homogenate: 1

Note: The diluent is extraction solution. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

Perform chromogenic reaction for exactly 5 minutes. Avoid excessive chromogenic reaction time.

9. Operating steps

1. Standard tubes: Add 20 μL of standards with different concentrations into the corresponding EP tubes. Control tubes: Add 20 μL of sample into the corresponding EP tubes. Sample tubes: Add 20 μL of sample into the corresponding EP tubes.
2. Add 80 μL of buffer solution to the standard tubes and control tubes. Add 80 μL of substrate working solution to the sample tubes.
3. Mix thoroughly and incubate at 37°C for 30 minutes.
4. Add 100 μL of chromogenic agent to each tube.
5. Mix thoroughly and place in a boiling water bath for 5 minutes. Cool the tubes to room temperature with running water.
6. Add 400 μL of ultrapure water to each tube.
7. Mix thoroughly and centrifuge at 10,000 × g for 10 minutes at 4°C.
8. Take 200 μL of supernatant into the wells. Measure the OD values of each well at 540 nm with microplate reader. Calculate ΔA = A_sample - A_control.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This gives the absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Plant tissue sample (Calculate for sample protein):

Definition: The amount of enzyme in 1 mg plant tissue protein that catalyzes the substrate to produce 1 µg sucrose in 1 minute at 37°C is defined as 1 unit.

2. Plant tissue sample (Calculate for sample weight):

Definition: The amount of enzyme in 1 g plant tissue that catalyzes the substrate to produce 1 µg sucrose in 1 minute at 37°C is defined as 1 unit.

Formulas:

SS-I activity (U/mgprot) = $33.33 \times (\Delta A - b)/a \div C_{pr} \times f$

SS-I activity (U/g wet weight) = $33.33 \times (\Delta A - b)/a \div m \times f \times V_2$

Where:

ΔA: $OD_{sample} - OD_{control}$

V_1: The volume of sample, mL

V_2: The volume of extracting solution, mL

T: Reaction time, 30 min

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

m: The wet weight of sample, g

10^3: 1 mg = 10^3 µg

f: Dilution factor of sample before test

11. Performance Characteristics

Parameters:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	15	27	51
%CV	5.4	4.3	3.7

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	10	15	26
%CV	9.2	9.1	5.1

Recovery

Three samples of high, medium, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 101%.

Recovery

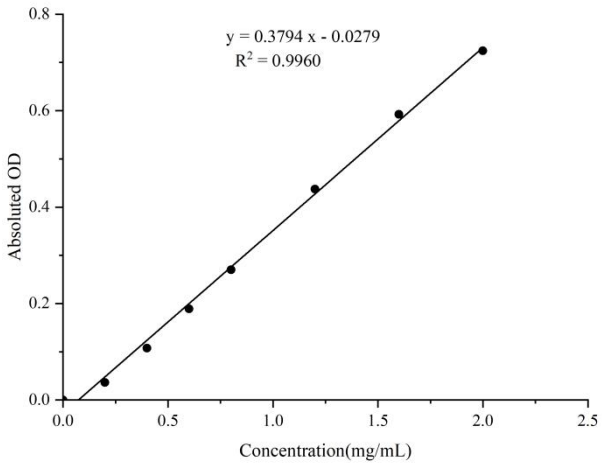
Sample	Expected Conc. (U/mL)	Observed Conc. (U/mL)	Recovery rate (%)
Sample 1	49.2	44.0	89%
Sample 2	51.2	54.4	106%
Sample 3	26.4	28.4	108%

Sensitivity

The analytical sensitivity of the assay is 0.39 U/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.

Standard curve data

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mg/mL)	OD	Average OD	Absolute OD
0	0.067/0.069	0.068	0.000
0.2	0.104/0.104	0.104	0.036
0.4	0.175/0.176	0.176	0.108
0.6	0.260/0.254	0.257	0.189
0.8	0.343/0.333	0.338	0.270
1.2	0.504/0.507	0.506	0.438
1.6	0.665/0.656	0.661	0.593
2	0.789/0.795	0.792	0.724

12. Example Analysis

Example analysis:

Take 20 µL of 10% corn seed tissue homogenate into the wells and perform the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.3794x - 0.0279

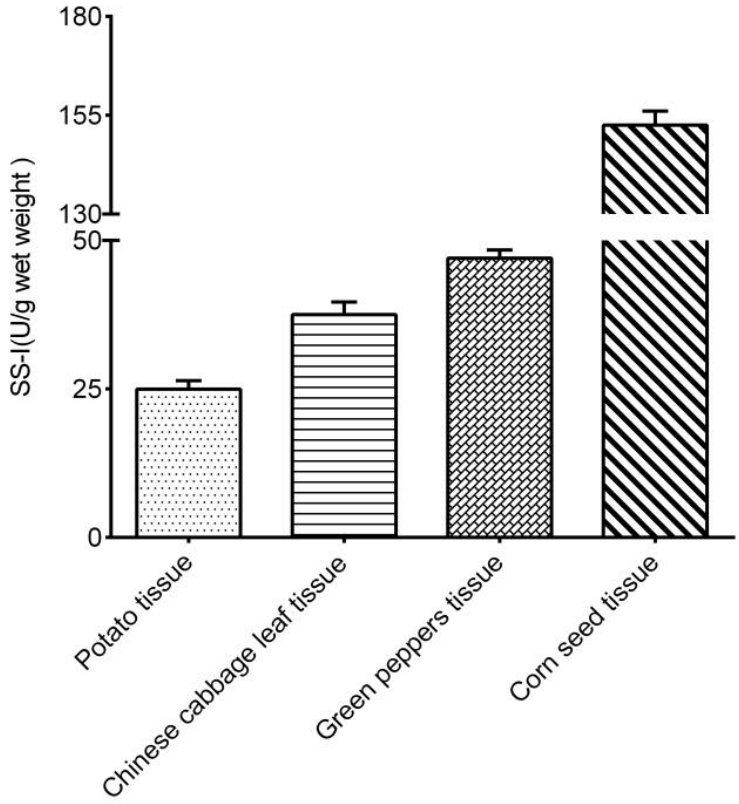
OD value of control wells: 0.408

OD value of sample wells: 0.572

Calculation result:

SS-I activity (U/g wet weight) = (0.572 - 0.408 + 0.0279) ÷ 0.3794 × 30 ÷ 0.1 = 151.74 U/g wet weight

Detection of 10% potato tissue homogenate, 10% Chinese cabbage leaf tissue homogenate, 10% green pepper tissue homogenate, and 10% corn seed tissue homogenate according to the protocol:



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.

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