TECHNICAL MANUAL

Sucrose Fluorometric Assay Kit

- **SKU CODE:** MAES0013
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and sample
- Incubation and enzymatic reaction
- Add reaction working solution
- Measure the fluorescence value

2. Intended use

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

3. 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 (horseradish 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.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	45 mL x 1 vial	-20 °C, 12 months
Reagent 2	Enzyme Reagent 1	Powder x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Buffer Solution	10 mL x 1 vial	-20 °C, 12 months
Reagent 4	Enzyme Reagent 2	Powder x 1 vial	-20 °C, 12 months, shading light
Reagent 5	Probe	0.25 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 6	Standard	Powder x 1 vial	-20 °C, 12 months

Item	Component	Size (96 T)	Storage
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/590 nm), Micropipettor, Incubator, Water bath, Centrifuge

6. Reagent preparation

1. Equilibrate all the reagents to room temperature before use.
2. **The preparation of extraction working solution::** Dilute 120 µL of extraction solution with 1080 µL of double distilled water and mix fully. Store at 2-8 °C for 7 days.
3. **The preparation of enzyme working solution 1::** Dissolve one vial of enzyme reagent 1 with 300 µL of double distilled water, mix well. Store at -20 °C for 7 days protected from light.
4. **The preparation of enzyme working solution 2::** Dissolve one vial of enzyme reagent 2 with 250 µL of double distilled water, mix well. Store at -20 °C for 7 days protected from light.
5. **The preparation of reaction working solution::** For each well, prepare 50 µL of reaction working solution (mix well 46 µL of buffer solution, 2 µL of enzyme working solution 2 and 2 µL of probe). The reaction working solution should be prepared on spot and protected from light.
6. **The preparation of 10 mmol/L standard::** Dissolve one vial of standard with 10 mL of double distilled water, mix well. Store at -20 °C for 7 days.
7. **The preparation of 100 µmol/L standard::** Dilute 10 µL of 10 mmol/L standard with 990 µL of extraction solution working solution and mix fully. Prepare the fresh solution before use.
8. **The preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µmol/L glucose standard with extraction solution working solution to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 5, 8, 10, 12, 15 µmol/L.

7. Standard dilution table

Item	1	2	3	4	5	6	7	8
Concentration (μmol/L)	0	1	2	5	8	10	12	15
100 μmol/L standard (μL)	0	5	10	25	40	50	60	75
Extraction solution working solution (μL)	500	495	490	475	460	450	440	425

8. Sample preparation

① 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 working solution with a dounce homogenizer at 4 °C.

③ Centrifuge at 12000 x g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ If not detected on the same day, the supernatant can be stored at -20 °C for 5 days.

② Dilution of sample

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

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

9. The key points of the assay

1. The volume of enzyme reagent 1 must be strictly controlled, otherwise it will produce a large error.
2. Fluorescent probe reaction must be performed with light protection.

10. Operating steps

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.:** Add 50 μL of sample into the sample and control wells. Add 50 μL of extraction working solution into the blank wells.
3. Mix fully with microplate reader for 10 s and incubate at 37 °C for 15 min.
4. Add 50 μL of reaction working solution into each well.
5. 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.

11. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absolute fluorescence value ($F_{Standard} - F_0$, F_0 is the fluorescence value when the standard concentration is 0).

3. Plot the standard curve by using absolute fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Sucrose content (μmol/g wet weight) = (ΔF - b) ÷ a × V × f ÷ W ÷ 1000

[Note]

ΔF: Absolute fluorescence intensity of sample, ΔF = (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 blank 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.

10^3: The coefficient of unit conversion.

12. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.00	7.00	13.00
%CV	2.5	2.2	2.2

Inter-assay Precision

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

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.00	7.00	13.00

Parameters	Sample 1	Sample 2	Sample 3
%CV	6.4	6.8	6.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 96%.

Recovery Results

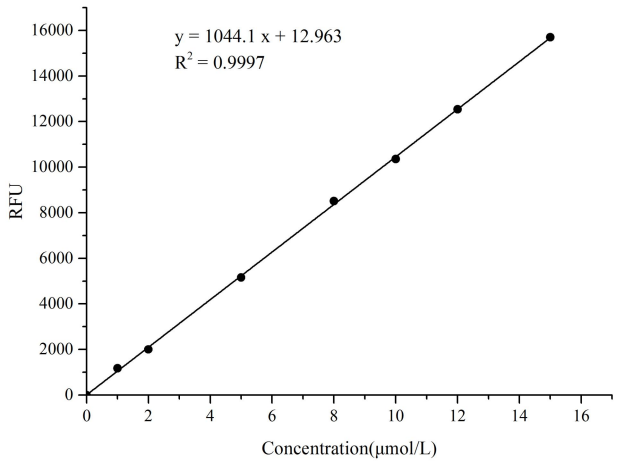
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	1.5	7.5	10
Observed Conc. (µmol/L)	1.5	7.1	9.5
Recovery rate (%)	98	95	95

Sensitivity

The analytical sensitivity of the assay is 0.15 µmol/L. 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

As the fluorescence 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 as below for reference only:



Standard curve data

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	3020	3130	3075	0
1	4234	4268	4251	1176
2	4953	5212	5083	2008
5	8142	8319	8231	5156
8	11568	11609	11589	8514
10	13374	13484	13429	10354
12	15512	15714	15613	12538
15	18765	18776	18771	15696

13. Appendix II Example Analysis

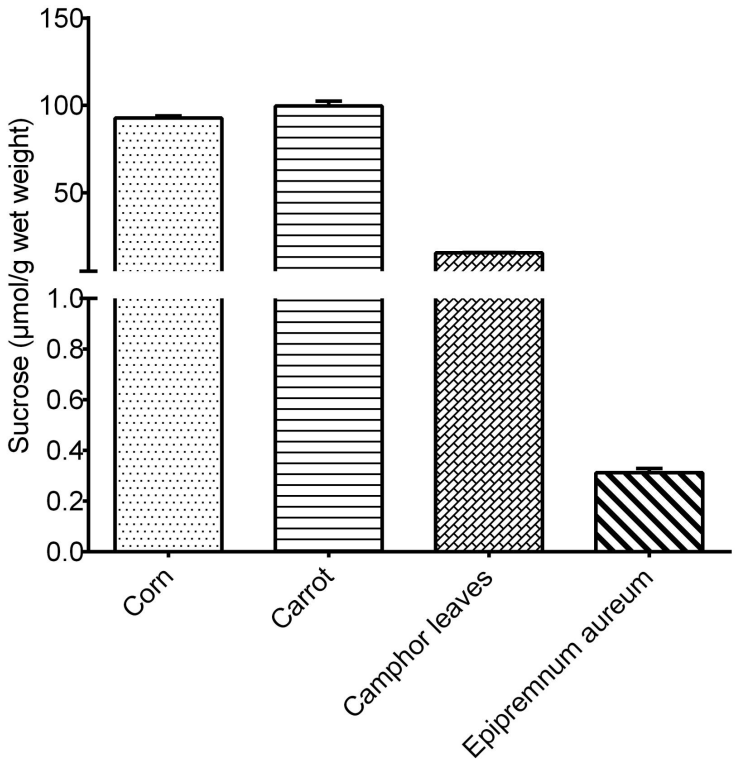
Example analysis:

For 10% corn grain tissue homogenate, take 2.5 μL of sample supernatant diluted for 1500 times and carry the assay according to the operation table. The results are as follows:

standard curve: y = 1044.1x + 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 μmol/g wet weight

Detect 10% corn tissue homogenate (dilute for 1500 times), 10% carrot tissue homogenate (dilute for 1500 times), 10% camphor leaves tissue homogenate (dilute for 150 times) and 10% epipremnum aureum tissue homogenate (dilute for 25 times) according to the protocol, the result is as follows:



14. 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 pre-test 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.