（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F042

Specification：96T(39 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/590 nm)

Detection range：0.15-15 μmol/L

Elabscience® Sucrose Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tel: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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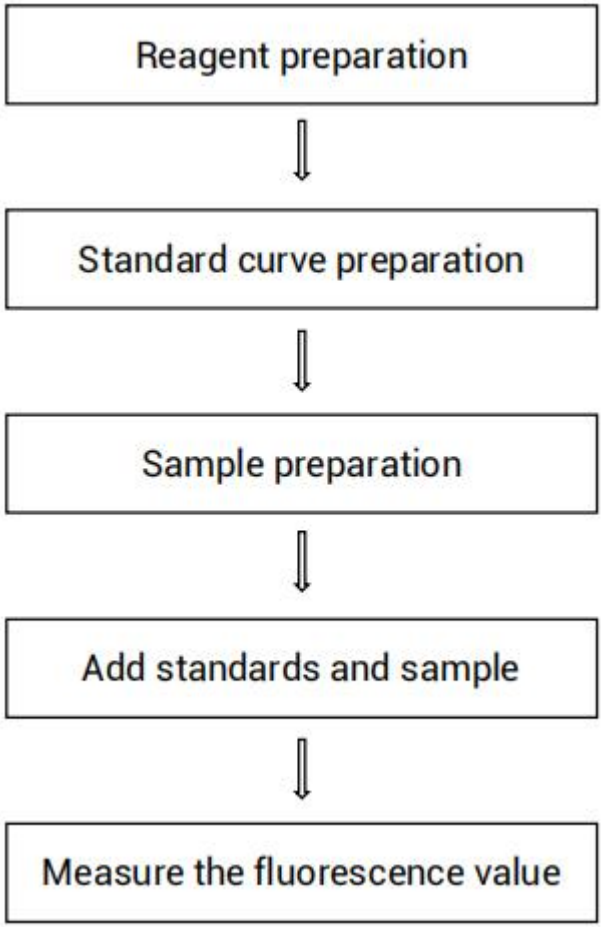
Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	45 mL × 1 vial	-20℃, 12 months
Reagent 2	Enzyme Reagent 1	Powder × 1 vial	-20℃, 12 months shading light
Reagent 3	Buffer Solution	10 mL × 1 vial	-20℃, 12 months
Reagent 4	Enzyme Reagent 2	Powder × 1 vial	-20℃, 12 months shading light
Reagent 5	Probe	0.25 mL × 1 vial	-20℃, 12 months shading light
Reagent 6	Standard	Powder × 1 vial	-20℃, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

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 uL 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 μmo/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. Reference is as follows:

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

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℃.

③ Centrifuge at 12000×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℃ for 5 days.

② **Dilution of sample**

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

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 with shading light.

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℃ 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℃ for 30 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

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 absoluted 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 absoluted 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: Absoluted fluorescence intensity of sample, $\Delta F = (F - F_0) - (F' - F_0')$.

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.

10^3: The coefficient of unit conversion.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three tomato 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 (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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	1.00	7.00	13.00
%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 parallelly to get the average recovery rate of 96%.

	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

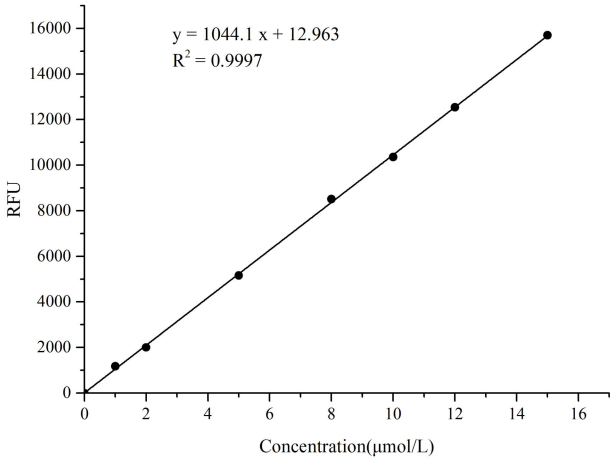
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only：

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



Appendix Π Example Analysis

Example analysis：

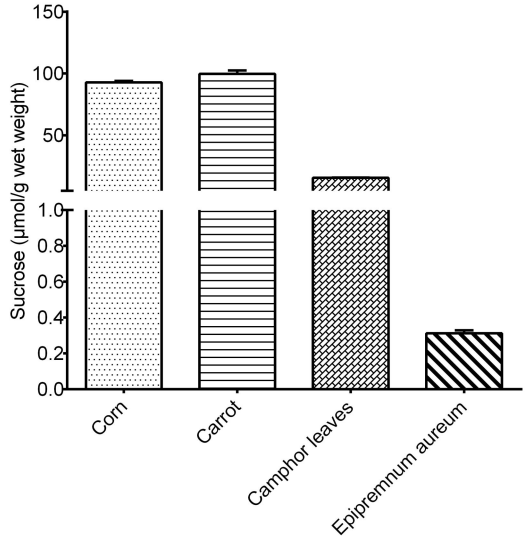
For 10% corn grain tissue homogenate, take 2.5 μL of sample supernatant diluted for 1500 times and carry the assay according to the operation table. The results are as follows:

standard curve: y = 1044.1 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

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：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.