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

Catalog No：E-BC-K1208-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(530-550 nm)

Detection range：0.39-66.67 U/mL

Elabscience® Sucrose Synthase-Ⅰ (SS-Ⅰ) Activity Colorimetric 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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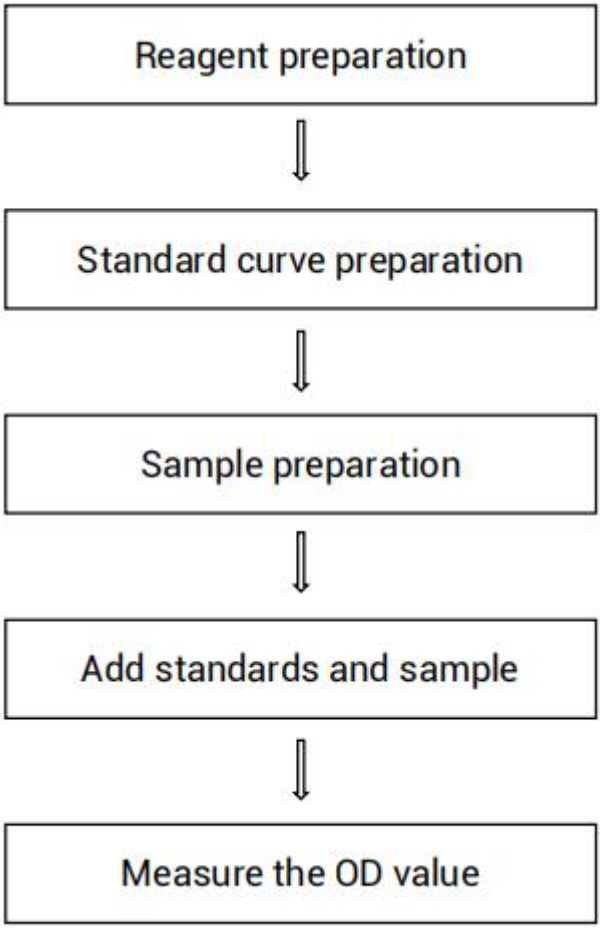
Assay summary



Intended use

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

Detection principle

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

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	55 mL×1 vial	-20℃, 12 months
Reagent 2	Buffer Solution	10 mL × 1 vial	-20℃, 12 months
Reagent 3	Substrate	Powder × 2 vials	-20℃, 12 months
Reagent 4	Chromogenic Agent	11 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	Standard	Powder × 2 vials	-20℃, 12 months
	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:

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

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate working solution：

Dissolve one vial of substrate with 2 mL of buffer solution, mix well. Aliquoted storage at -20°C for a week.

③ Before using chromogenic agent, water bath at 60-70℃ for 10 min.

④ The preparation of 2 mg/mL standard solution：

Dissolve one vial of standard with 5 mL of extraction solution, mix well. Aliquoted storage at -20°C for a week.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 2 mmol/L standard solution with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.2**	**1.6**	**2**
2 mg/mL standard (μL)	0	20	40	60	80	120	160	200
Extraction solution (μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation

Tissue sample:

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

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

③ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

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

Sample type	Dilution factor
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 the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Chromogenic reaction for 5 min accurately, avoid excessive chromogenic reaction time.

Operating steps

① Standard tube: add 20 μL of standards with different concentrations into the corresponding EP tubes.

Control tube: add 20 μL of sample into the corresponding EP tubes.

Sample tube: 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 fully, incubate at 37℃ for 30 min.

④ Add 100 μL of chromogenic agent to each tube.

⑤ Mix fully, boiling water bath for 5 min. Cool the tubes to room temperature with running water.

⑥ Add 400 μL of ultrapure water to each tube.

⑦ Mix fully, centrifuge at 10000 × g for 10 min at 4℃

⑧ Take 200 μL of supernatant into the wells. Measure the OD values of each well at 540 nm with microplate reader, $\Delta A = A_{sample} - A_{control}$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD 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:

1. **The plant tissue sample (Calculate for sample protein)**

   Definition: The amount of enzyme in 1 mg plant tissue protein that catalyze the substrate to 1 μg sucrose in 1 min at 37℃ is defined as 1 unit.

$$\underset{(\mathrm{U/mgprot})}{\text{SS-Ⅰ activity}} = \frac{\Delta A - b}{a} \times V_1 \times 10\text{^}3 \div T \div (V_1 \times Cpr) \times f = 33.33 \times \frac{\Delta A - b}{a} \div Cp$$

2. **The plant tissue sample (Calculate for sample weight)**:

   Definition: The amount of enzyme in 1 g plant tissue that catalyze the substrate to 1 μg sucrose in 1 min at 37℃ is defined as 1 unit.

$$\underset{(\mathrm{U/g\ wet\ weight})}{\text{SS-Ⅰ activity}} = \frac{\Delta A - b}{a} \times V_1 \times 10\text{^}3 \div T \div (V_1 \div V_2 \times m) \times f = 33.33 \times \frac{\Delta A - b}{a} \div m \times f$$

[Note]

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

Cpr: 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.

Appendix I Performance Characteristics

1. Parameter:

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).

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.

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

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 101%.

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

Sensitivity

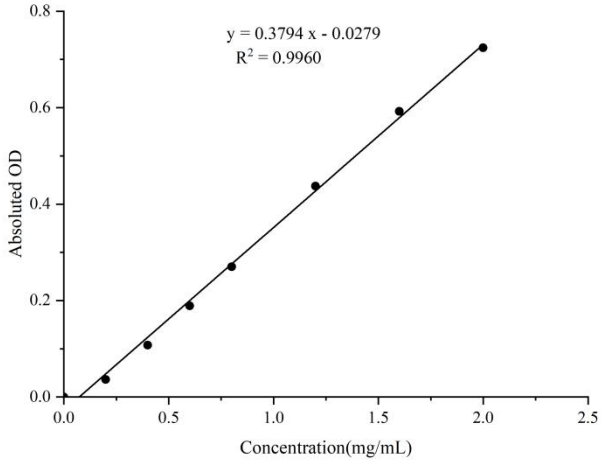
The analytical sensitivity of the assay is 0.39 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the

zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

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

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



Appendix Π Example Analysis

Example analysis：

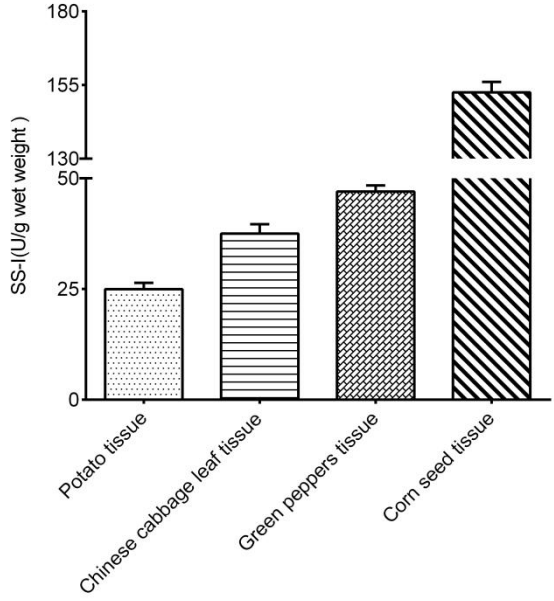
Take 20 μL of 10% corn seed tissue homogenate into the wells and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.3794 x – 0.0279, the OD value of control wells is 0.408, the OD value of the sample wells is 0.572, and the calculation result is:

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

= 151.74 U/g wet weight

Detect 10% potato tissue homogenate, 10% chinese cabbage leaf tissue homogenate, 10% green peppers tissue homogenate, 10% corn seed tissue homogenate 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.