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

Catalog No：E-BC-K1207-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(470 - 490 nm)

Detection range：110 - 4000 U/g wet weight

Elabscience® Sucrose Synthase-Ⅱ (SS-Ⅱ) 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
Tell: 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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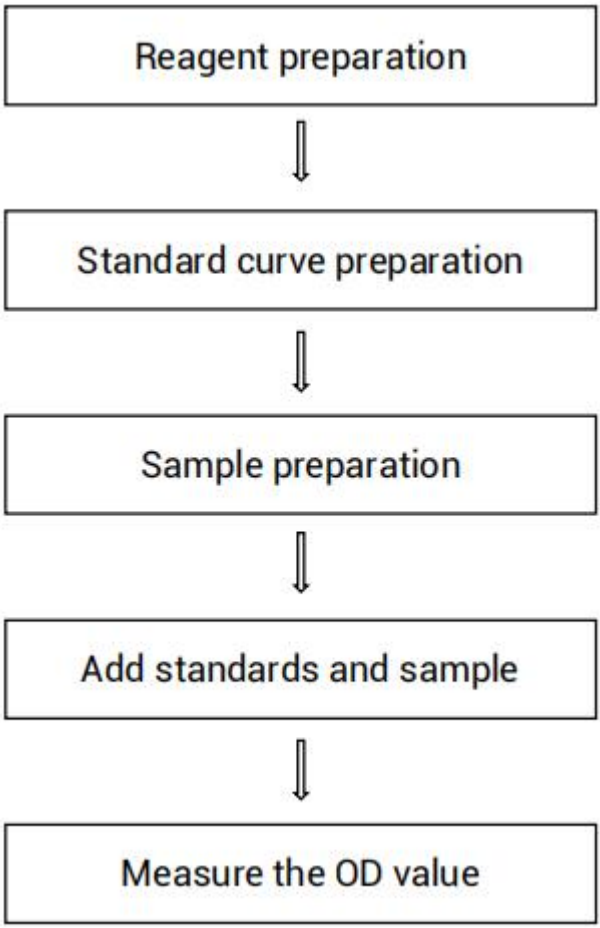
Assay summary



Intended use

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

Detection principle

Sucrose synthase-Ⅱ (SS-Ⅱ) catalyzes the reaction of substrate to produce sucrose, which reacts with chromogenic agents to produce chromogenic substances. The characteristic absorption peak at 480 nm is found, and the enzyme activity is proportional to the color.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	60 mL × 1 vial	-20°C, 12 months
Reagent 2	Reaction Solution A	9 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Reaction Solution B	11 mL × 1 vial	-20°C, 12 months
Reagent 4	Reaction Solution C	33 mL × 2 vials	-20°C, 12 months
Reagent 5	Chromogenic Agent	14 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Standard	Powder × 2 vials	-20°C, 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 (470 - 490 nm, optimum wavelength: 480 nm), Homogenizer, Constant temperature water bath, Incubator, Centrifuge.

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of 10 mg/mL standard solution：

Dilute one vial of standard with 1 mL of extraction solution. Store at 2-8°C for a week.

③ The preparation of standard curve：

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

Dilute 10 mg/mL standard solution with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.25**	**0.50**	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**
10 mg/mL Standard (μL)	0	10	20	40	80	120	160	200
Extraction solution (μL)	400	390	380	360	320	280	240	200

Sample preparation

① Sample preparation

Tissue sample:

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution water with a dounce homogenizer at 4°C.

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

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

Control sample:

Take 0.3 mL of supernatant for detection to the new EP tubes, bath in water for 5 min, cool down with running water, as control sample.

② Dilution of sample

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

Sample type	Dilution factor
5% Corn seed tissue homogenate	1-5
5% Potato tissue homogenate	1
5% Sweet potato tissue homogenate	1-5
5% Green dill leaf 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

① When preparing 10 mg/mL standard solution, ensure that the powder is completely dissolved.

② The chromogenic agent is volatile, and the cap should be tightened during storage.

Operating steps

① Standard tube: Add 40 μL of standard solution with different concentrations into the corresponding EP tubes.
Sample tube: Add 40 μL of sample into the corresponding EP tubes.
Control tube: Add 20 μL of control sample into the corresponding EP tubes.

② Add 80 μL of ultrapure water into standard tubes.
Add 80 μL of reaction solution A into sample and control tubes.

③ Mix fully with microplate reader. Incubate at 37°C for 20 min

④ Add 100 μL of reaction solution B to each tube.

⑤ Mix fully, water bath for 10 min and then cool down with running water.

⑥ Add 600 μL of reaction solution C and 120 μL of chromogenic agent into each tube.

⑦ Mix fully, water bath for 10 min and then cool down with running water. Centrifuge at 10000 × g for 10 min.

⑧ Take 300 μL of supernatant. Measure the OD value of each well at 480 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.

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

$$= 50 \times \frac{\Delta A - b}{a} \div Cpr \times f$$

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.

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

$$= \frac{\Delta A - b}{a} \times 50 \div m \times V_2 \times f$$

[Note]

ΔA: $\Delta A = OD_{Sample} - OD_{Control}$.

V_1: The volume of sample, 0.04 mL

V_2: The volume of extraction solution added to the reaction system, mL

T: Reaction time, 20 min

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

m: The 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 potato 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 (U/g wet weight)	5.0	2.5	1.0
%CV	2.0	1.8	1.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g wet weight)	5.0	2.5	1.0
%CV	3.5	2.0	1.9

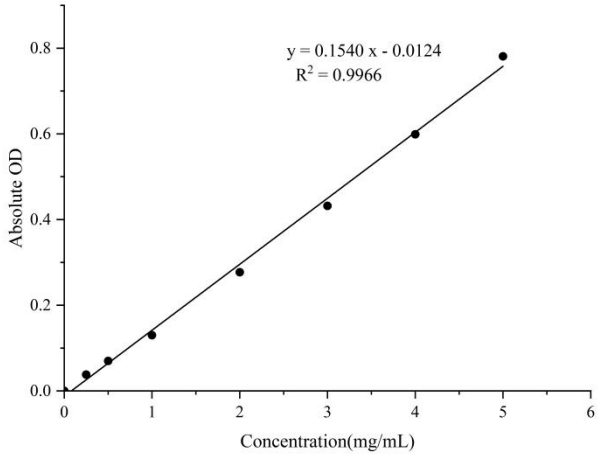
Sensitivity

The analytical sensitivity of the assay is 30 U/g wet weight. 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	0.25	0.50	1.00	2.00	3.00	4.00	5.00
OD Value	0.051	0.090	0.123	0.177	0.324	0.476	0.638	0.826
	0.048	0.084	0.116	0.181	0.328	0.486	0.658	0.835
Average OD	0.050	0.087	0.120	0.179	0.326	0.481	0.648	0.831
Absoluted OD	0.000	0.038	0.070	0.130	0.277	0.432	0.599	0.781



Appendix Π Example Analysis

Example analysis：

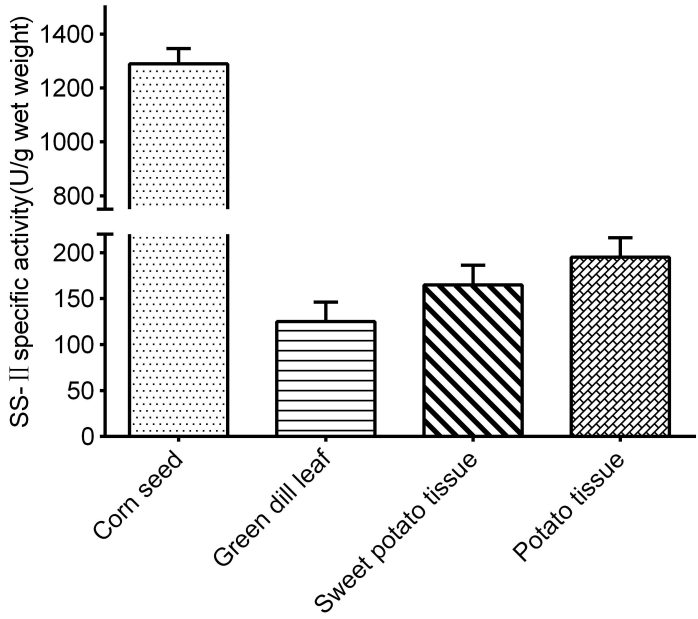
Take 40 μL of 5% corn seed tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.154 x - 0.0124, the OD value of the control is 1.227, the OD value of the sample is 1.448, and the calculation result is:

SS-Ⅱ activity (U/g wet weight) = (1.448 - 1.227 + 0.0124) ÷ 0.154 × 50 ÷ 0.05 × 0.95

= 1439.80 U/g wet weight

Detect 5% corn seed tissue homogenate, 5% green dill leaf tissue homogenate, 5% sweet potato tissue homogenate, 5% potato 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.