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

Catalog No：E-BC-K1204-M

Specification：48T(24 samples)/96T(48 samples)

Measuring instrument：Microplate reader（650-670 nm）

Detection range：1.5-15.0 U/g wet tissue

Elabscience® Starch Branching Enzyme (SBE) 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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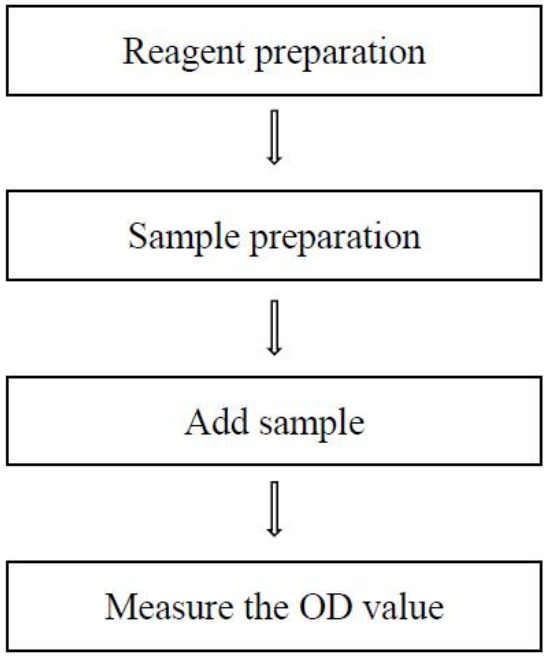
Assay summary



Intended use

This kit can be used to measure starch branching enzyme (SBE) activity in plant tissue samples.

Detection principle

Starch Branching Enzyme (SBE) catalyzes the conversion of Amylose to Amylopectin. SBE can reduce the content of amylose and thus reduce the optical absorption value of starchiodine complex at 660 nm. The percentage of absorbance decline in a certain time can reflect the activity of SBE.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months shading light
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 3	Stop Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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 (650-670 nm, optimum wavelength: 660 nm), Water bath

Reagent preparation

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

② The preparation of substrate working solution：

Dilute one vial of substrate with 3 mL of double distilled water, water bath at 95°C until complete dissolution. Store at 2-8°C for a month, water bath at 95°C was required for 10 min before each use.

Sample preparation

① Sample preparation

Plant 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℃.

③ Centrifuge at 10000×g for 10 minutes 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).

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
10% Corn tissue homogenate	2-5
10% Red been tissue homogenate	5-10
10% Peanut tissue homogenate	5-15
10% Mung been tissue homogenate	5-10

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

The chromogenic product will settle for a long time, so it is recommended that no more than 20 tubes should be detected at one time and determined immediately after chromogenic development.

Operating steps

① Sample tube: Add 100 μL of sample into corresponding tubes.
Control tube: Add 100 μL of control sample into corresponding tubes.

② Add 50 μL of substrate working solution into each tubes.

③ Mix well with the vortex mixer, incubate accurately at 37°C for 30 min, incubate at 95°C water bath for 5 min. Then cool down with running water.

④ Add 100 μL of stop solution into each tube.

⑤ Add 50 μL of chromogenic agent into each tube, mix fully immediately.

⑥ Take 200 μL of the solution in step 5 into the microplate and measure the OD value of each well at 660 nm.

Calculation

The plant tissue sample (Calculate for sample weight):

Definition: The percentage decrease in absorbance at a wavelength of 660 nm by 1% decrease in iodine-blue value 1 g of tissue sample per minute in the reaction system at 37°C that is defined as an enzyme activity unit.

$$\text{SBE activity (U/g wet tissue)} = (A_{control} - A_{sample}) \div A_{control} \times 100\% \div 1\% \div T \times f \div \frac{m}{V}$$

The plant tissue sample (Calculate for protein concentration):

Definition: The percentage decrease in absorbance at a wavelength of 660 nm by 1% decrease in iodine-blue value 1 mg of protein sample per minute in the reaction system at 37°C that is defined as an enzyme activity unit.

$$\text{SBE activity (U/mgprot)} = (A_{control} - A_{sample}) \div A_{control} \times 100\% \div 1\% \div T \times f \div C_{pr}$$

[Note]

$A_{control}$: The OD value of control well

A_{sample}: The OD value of sample well

T: Reaction time, 30 min;

f: The dilution factor of sample before tested

m: The weight of sample, g

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

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

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 tissue)	2.7	4.7	7.8
%CV	4.6	4.1	2.2

Inter-assay Precision

Three potato tissue samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g wet tissue)	2.7	4.7	7.8
%CV	4.8	3.8	3.3

Sensitivity

The analytical sensitivity of the assay is 1.5 U/g wet tissue. 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.

Appendix II Example Analysis

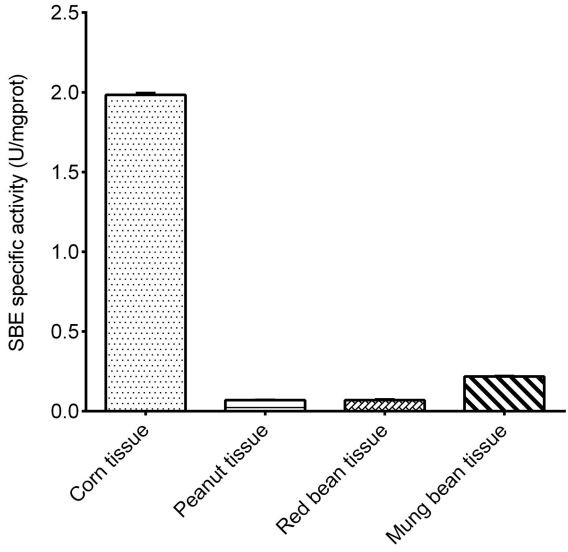
Example analysis:

Take 100 μL of 10% corn tissue homogenate, dilute for 2 times as sample and 100 μL of inactivated sample, and carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample is 0.543, the average OD value of the control is 0.811, $\triangle A = A_{control} - A_{sample} = 0.811 - 0.543 = 0.268$, the concentration of 10% corn tissue homogenate is 1.11 mgprot/mL, and the calculation result is:

$$\text{SBE activity (U/mgprot)} = (0.811 - 0.543) \div 0.811 \times 100\% \div 1\% \div 30 \times 2 \div 1.11 = 1.98 \text{ U/mgprot}$$

Detect 10% corn tissue homogenate (dilute for 2 times, the concentration of protein is 1.11 mgprot/mL), 10% peanut tissue homogenate (dilute for 5 times, the concentration of protein is 12.44 mgprot/mL), 10% red been tissue homogenate (dilute for 5 times, the concentration of protein is 11.16 mgprot/mL), 10% mung been tissue homogenate (dilute for 5 times, the concentration of protein is 11.29 mgprot/mL) 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.