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

Catalog No：E-BC-K1206-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（530-550 nm）

Detection range：0.9-18.0 U/g

Elabscience® Starch Debranching Enzyme (DBE) 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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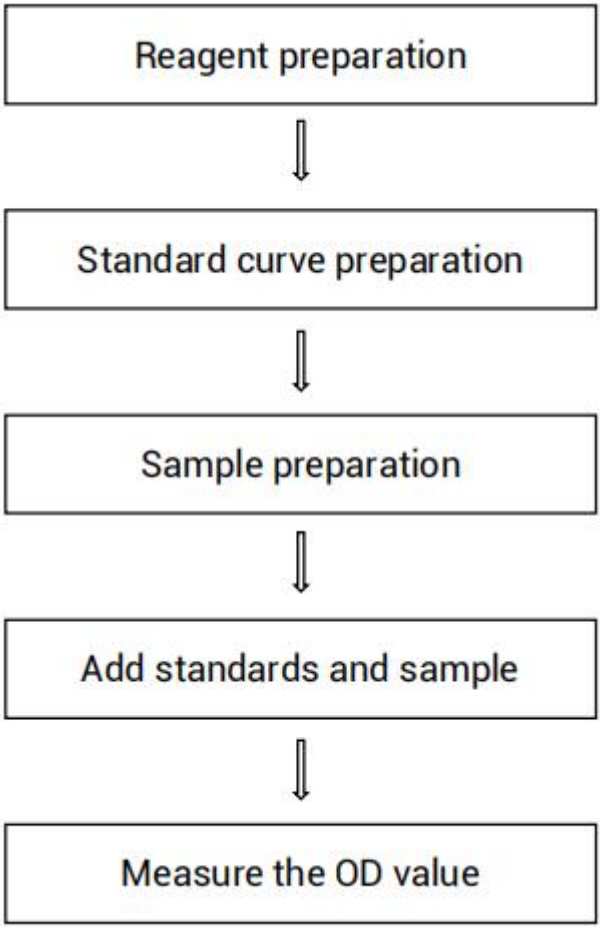
Assay summary



Intended use

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

Detection principle

Starch Debranching Enzyme (DBE) can specifically split the α-1, 6-glucoside bonds of amylopectin. The resulting reducing sugars were determined by 3, 5-dinitrosalicylic acid method, and their light absorption values were measured at
540 nm. Then the activity of starch debranching enzyme was calculated indirectly.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 2	Substrate	Power × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Stop Solution	8 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Chromogenic Agent	24 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 5	Standard	Power × 1 vial	2-8°C, 12 months, shading light
	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 (37℃), Water bath.

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② Before sample testing, chromogenic agent should be heated in a 75℃ water bath for 10 min, and then cooled to 25℃ with running water.

③ The preparation of substrate working solution:
Dissolve one vial of substrate with 4 mL of double distilled water, mix it in water bath at 95°C until completely dissolved. Store at 2-8°C for 1 month. Before using, incubute the prepared solution at 95°C for 10 min.

④ The preparation of 10 mg/mL standard solution:
Dissolve one vial of standard with 1 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 1 month.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 10 mg/mL standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0 mg/mL. Reference is as follows:

Item	①	②	③	④	⑥	⑧	⑧	⑧
Concentration (mg/mL)	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.2**	**1.6**	**2.0**
10 mg/mL standard (μL)	0	10	20	30	40	60	80	100
Double distilled water (μL)	500	490	480	470	460	440	420	400

Sample preparation

① **Sample preparation:**

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation
0.1 g).

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

③ Homogenize 0.1 g tissue in 0.9 mL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 15000 × 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).

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	2-8
10% Peanut tissue homogenate	2-4

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Take part of the sample supernatant, and inactivate it in a 95°C water bath for 5 min (After the sample is cooled, there may be precipitation, no need to centrifuge, just mix fully).

② Standard tube: Add 60 μL of standard solution with different concentrations to the corresponding tube.

Sample tube: Add 60 μL of sample to the corresponding tube.

Control tube: Add 60 μL of inactivate sample to the corresponding tube.

③ Add 60 μL of substrate working solution to each tube.

④ Mix fully, incubate at 37°C for 1 h.

⑤ Add 60 μL of stop solution to each tube.

⑥ Add 180 μL of chromogenic agent to each tube.

⑦ Mix fully, water bath at 95°C for 5 min, then cooled to with running water.

⑧ Centrifuge the sample tube and control tube at 15000×g for 5 min at 25℃

⑨ Take 200 μL of supernatant of each tube to the microplate. Measure the OD value of each well at 540 nm with microplate reader (without centrifugation, directly add 200 μL of the solution of standard tube to microplate).

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:

Tissue samples (Calulate for sample weight):

Definition: The amount of enzyme in 1 g of tissue that produce 1 mg reducing sugar at 37 ℃ for 1 h is defined as 1 unit.

$$\underset{(U/g)}{\text{DBE acticity}} = (\Delta A - b) \div a \div T \times f \div \frac{m}{V}$$

Tissue samples (Calulate for protein concentration):

Definition: The amount of enzyme in 1 mg of tissue protein that produce 1 mg reducing sugar at 37 ℃ for 1 h is defined as 1 unit.

$$\underset{(U/mgprot)}{\text{DBE activity}} = (\Delta A - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

T: Reaction time, 1 h

f: Dilution factor of sample before test.

m: The weight of sample, g

V: The volum of extraction solution, mL

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three corn 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)	4.5	9.0	16.2
%CV	0.7	1.2	0.7

Inter-assay Precision

Three corn 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)	4.5	9.0	16.2
%CV	1.2	1.0	1.4

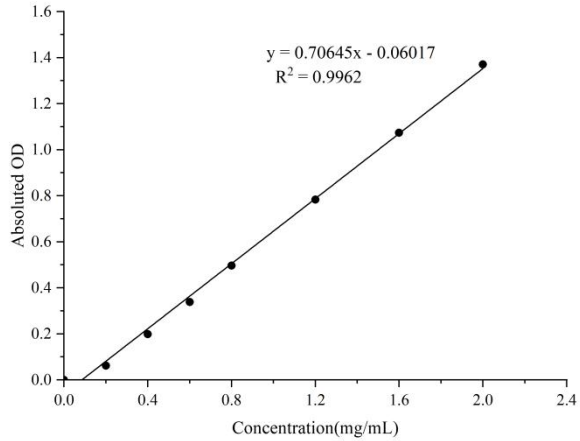
Sensitivity

The analytical sensitivity of the assay is 0.9 U/g. 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	2.0
OD	0.114	0.177	0.316	0.439	0.610	0.878	1.195	1.493
	0.114	0.175	0.310	0.465	0.611	0.916	1.180	1.476
Average OD	0.114	0.176	0.313	0.452	0.611	0.897	1.188	1.485
Absoluted OD	0.000	0.062	0.199	0.338	0.497	0.783	1.074	1.371



Appendix Π Example Analysis

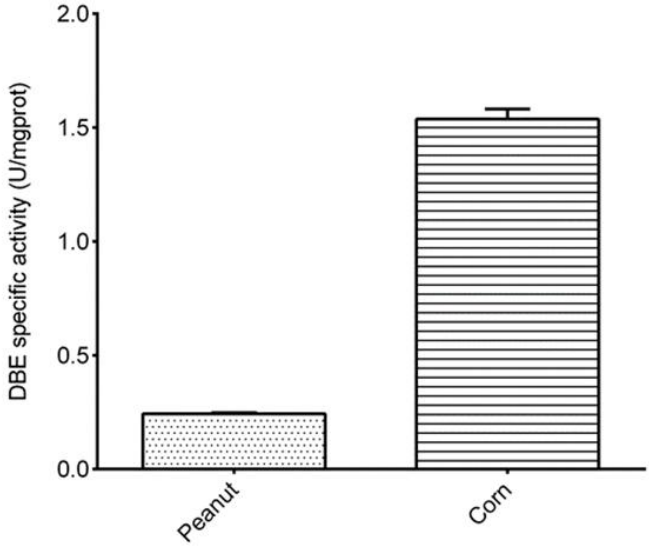
Example analysis：

Take 60 μL of 10% peanut tissue homogenate supernatant (diluent for 2 times) and inactivated sample, carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.7065 x - 0.0602, the average OD value of the sample well is 0.566, the average OD value of the control well is 0.356, ΔA = 0.566 - 0.356 = 0.210, the concentration of protein in sample is 3.15 mgprot/mL, and the calculation result is:

$$\text{DBE activity (U/mgprot)} = (0.210 + 0.0602) \div 0.7065 \div 1 \times 2 \div 3.15 = 0.24 \text{ U/mgprot}$$

Detect 10% peanut tissue homogenate (the concentration of protein is 3.15 mgprot/mL), 10% corn tissue homogenate (the concentration of protein is 0.79 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.