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

Catalog No：E-BC-K005-M

Specification：96T(20 samples)/500Assays(121 samples)

Measuring instrument：Microplate reader（535-540 nm）

Detection range：0.97-34.74 U/g tissue

Elabscience® β-Amylase Activity 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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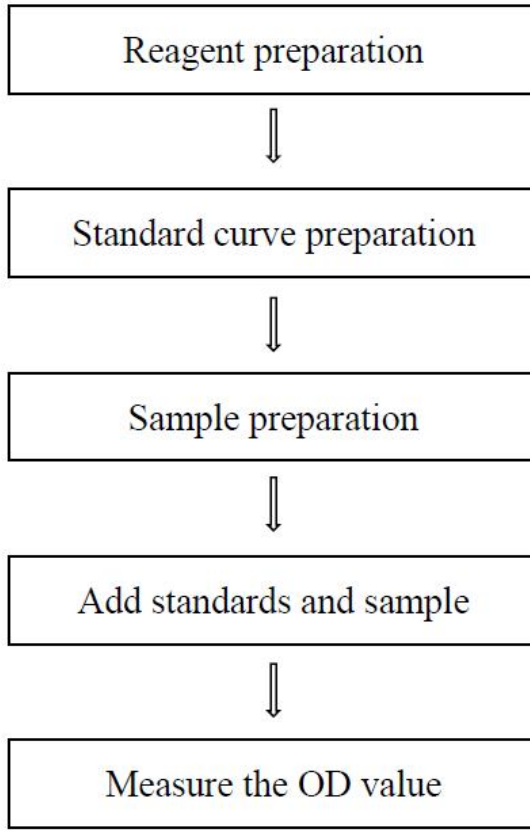
Assay summary



Intended use

This kit can measure β-Amylase activity in plant tissue samples.

Detection principle

The reducing sugar reacts with 3,5-dinitrosalicylic acid under heating conditions to produce a brown-red substance. β-amylase was inactivated by the property of amylase not to be heat-resistant, and then the enzyme activity of total amylase and α-amylase is determined. So the activity of β-amylase can be calculated indirectly.

Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500Assays)	Storage
Reagent 1	Substrate	10 mL × 1 vial	50 mL × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent	20 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 3	10 mg/mL Standard	1.5 mL × 1 vial	7.5 mL × 1 vial	2-8°C, 12 months
	Microplate	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:

Test tubes, Vortex Mixer, Centrifuge, Water bath, Microplate reader (540 nm）

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② If there is precipitation in substrate and chromogenic agent, Heat it at 70-80°C in water bath until dissolved. Cool down to 40°C with fresh water before use.

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 double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 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**	**1.0**	**1.2**	**1.4**
10 mg/mL standard (μL)	0	20	40	60	80	100	120	140
Double distilled water (μL)	1000	980	960	940	920	900	880	860

Sample preparation

① Sample preparation

Tissue sample:

① Weigh 0.1 g sample, add 0.9 mL of distilled water and homogenized with a homogenizer in ice water bath, then transfer to the EP tube, incubate at room temperature for 15 min and oscillate every 5 min.

② Centrifuge at 3000×g at room temperature for 10 min, take the supernatant and add double distilled water to the final volume of 10 mL, mix fully and it is the α-amylase solution.

③ Take 1 mL amylase solution and add 4 ml of distilled water, mix fully to prepare diluted amylase solution which is for the measurement of (α+β) amylase activity.

② Dilution of sample

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

Sample type	Dilution factor
1% Epipremnum aureum tissue homogenate	1
1% Green pepper tissue homogenate	1
1% Corn grain tissue homogenate	1
1% Daucus carota tissue homogenate	1

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

The key points of the assay

① For measuring the OD value, if there is precipitation, centrifuge at 4000 g for 5 min at room temperature and take the supernatant for determination.

② If the amylase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K168-M，E-BC-K168-S).

③ When the absolute OD value is more than 0.747, it is recommended to dilute the sample appropriately.

Operating steps

1. The measurement of standard

① Take 1.5 mL EP tube and number the tubes from A to H in duplication, add 75 μL of standard solution with different concentrations to the corresponding tubes.

② Add 75 μL of substrate to each tube.

③ Add 150 μL of chromogenic agent to each tube.

④ Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.

2. The measurement of α-amylase activity in sample (every sample tube need a control tube)

① Sample tube: add 75 μL of α-amylase solution to the corresponding tubes.
Control tube: add 75 μL of α-amylase solution to the corresponding tubes.

② Incubate at 70°C water bath for 15 min and cool the tubes with running water.

③ Sample tube: add 75 μL of substrate to the corresponding tubes.
Control tube: add 75 μL of double distilled water to the corresponding tubes.

④ Incubate the sample tubes and control tubes at 40°C water bath for 5 min.

⑤ Add 150 μL of chromogenic agent to each tube.

⑥ Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.

3. The measurement of (α+β) amylase activity in sample (every sample tube need a control tube)

① Sample tube: Add 75 μL of diluted amylase solution to the corresponding tubes.

Control tube: Add 75 μL of diluted amylase solution to the corresponding tubes.

② Sample tube: Add 75 μL of substrate to the corresponding tubes.

Control tube: Add 75 μL of double distilled water to the corresponding tubes.

③ Incubate the sample tubes and control tubes at 40°C water bath for 5 min.

④ Add 150 μL of chromogenic agent to each tube.

⑤ Mix fully and incubate at 95°C for 5 min. Cool the tubes with running water and take 250 μL of supernatant to the microplate. Measure the OD value of each well with microplate reader at 540 nm.

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. Calculate according to the protein concentration of the sample

Definition: The production of 1 mg reducing sugar catalyzed by 1 mg of tissue protein per minute that is defined as an enzyme activity unit.

$$\text{α-amylase activity (U/mgprot)} = (\Delta A - b) \div a \times V_3 \div t \div V_2 \div C_{pr} \times f$$

$$\text{(α+β) amylase activity (U/mgprot)} = (\Delta A - b) \div a \times V_3 \div t \div V_2 \times f \div C_{pr} \times 5^*$$

2. Calculate according to the fresh weight of sample

Definition: The production of 1 mg reducing sugar catalyzed by 1 g of tissue per minute that is defined as an enzyme activity unit.

$$\text{α-amylase activity (U/g fresh weight)} = (\Delta A - b) \div a \times V_3 \div t \div w \times \frac{V_1}{V_2} \times f$$

$$\text{(α+β) amylase activity (U/g fresh weight)} = (\Delta A - b) \div a \times V_3 \div t \div w \times \frac{V_1}{V_2} \times f \times 5^*$$

β-amylase activity =(α+β) amylase activity - α-amylase activity

[Note]

f: Dilution factor of amylase solution before tested.

ΔA: $OD_{Sample} - OD_{Control}$.

V_1: The volume of prepared tissue sample in sample preparation step (10 mL).

V_2: The volume of sample added to the reaction (0.075 mL).

V_3: The volume of enzymatic reaction (the volume of sample + the volume of substrate = 0.15 mL).

t: The time of enzymatic reaction (5 min).

w: The weight of tissue sample (0.1 g).

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

5*: Dilution factor for the preparation of diluted amylase solution.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three epipremnum aureum 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 (mg/mL)	1.50	15.00	25.00
%CV	2.6	2.0	2.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	1.50	15.00	25.00
%CV	3.0	3.4	3.2

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. (mg/mL)	0.3	0.7	1.1
Observed Conc. (mg/mL)	0.3	0.7	1.0
Recovery rate (%)	98	95	95

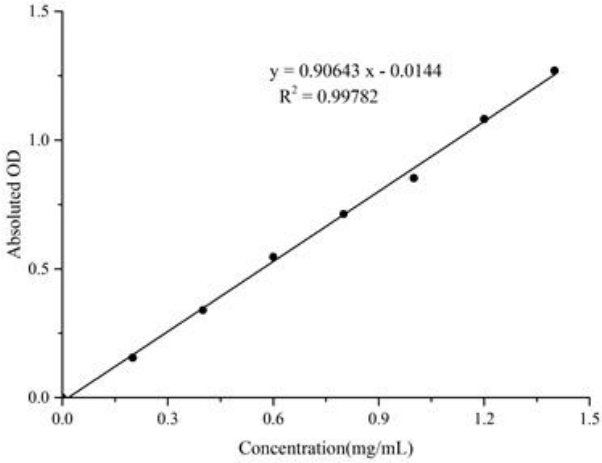
Sensitivity

The analytical sensitivity of the assay is 0.97 U/g 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.

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**	**1.0**	**1.2**	**1.4**
OD value	0.156	0.308	0.497	0.700	0.886	1.013	1.238	1.440
	0.155	0.313	0.494	0.704	0.851	1.003	1.236	1.411
Average OD	0.156	0.310	0.496	0.702	0.869	1.008	1.237	1.426
Absoluted OD	0	0.154	0.340	0.546	0.713	0.852	1.081	1.270



Appendix II Example Analysis

Example analysis:

Take 0.1 g of green pepper, treat the sample according to the sample preparation step, take 0.1 mL of α-amylase solution and add 0.4 mL of double distilled water, mix fully to prepare the diluted amylase solution and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.8729 x - 0.0112, the average OD value of the sample is 0.368, the average OD value of the control is 0.247, and the calculation result of α-amylase activity is:

$$\text{α-amylase activity (U/g fresh weight)} = (0.368 - 0.247 + 0.0112) \div 0.8729 \times 0.15 \div 5 \div 0.1 \times 10 \div 0.075$$

$$= 6.06 \text{ U/g fresh weight}$$

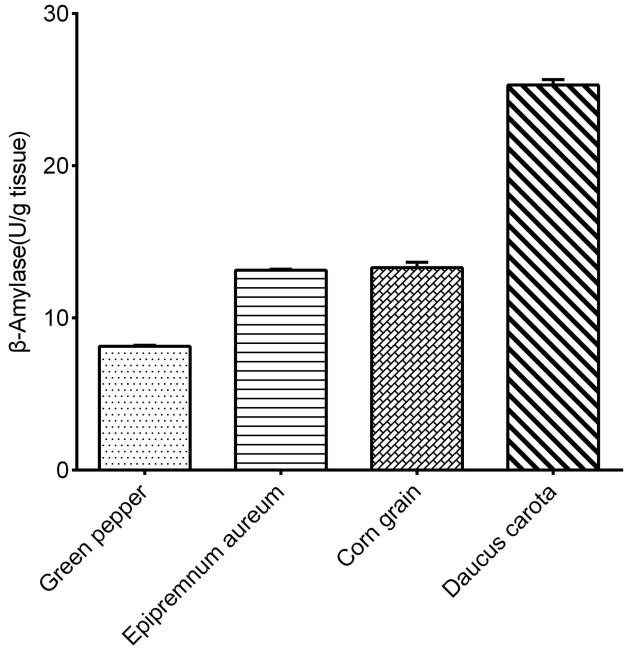
the calculation of (α+β) amylase activity: the average OD value of the sample is 0.205, the average OD value of the control is 0.154, and the calculation result is:

$$\text{(α+β) amylase activity (U/g fresh weight)} = (0.205 - 0.154 + 0.0112) \div 0.8729 \times 0.15 \div 5 \div 0.1 \times 10 \div 0.075 \times 5$$

$$= 14.25 \text{ U/g fresh weight}$$

$$\text{β-amylase activity (U/g fresh weight)} = 14.25 - 6.06 = 8.19 \text{ U/g fresh weight}$$

Detect green pepper, epipremnum aureum, corn grain and daucus carota 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.