β-Amylase Activity Assay Kit

Catalog No: E-BC-K005-M

Method: Colorimetric method

Specification: 96T (Can detect 20 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.97 U/g tissue

Detection range: 0.97-34.74 U/g tissue

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

β-Amylase is a non-metallic exo-amylase, which decomposes polyglucan at the α-1,4-glycosidic bond, releasing β-maltose and trace β-limit dextrin. β-Amylase is mainly distributed in higher plants and is also found in some microorganisms. In abiotic stress, the enzyme plays a key role in starch degradation, early seed germination and cell protection.

▲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	Specification	Storage
Reagent 1	Substrate	10 mL×1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent	20 mL×1 vial	2-8℃ , 6 months, shading light
Reagent 3	10 mg/mL Standard	1.5 mL×1 vial	2-8℃ , 6 months
	Microplate	96 wells	
	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.			

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲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-K318-M).
3. When the absolute OD value is more than 0.747, it is recommended to dilute the sample appropriately.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use. Before the experiment, preheat reagent 1 and reagent 2 at 40°C for 10 min.
2. If there is precipitation in reagent 1, please use it after heating and dissolving at 70°C.
3. If there is yellow precipitation in reagent 2, please use it after heating and dissolving at 70°C.

▲Sample preparation

Tissue sample:

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

(2) 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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.97-34.74 U/g tissue).

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.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	530 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	α1'	α1	α5'	α5	α9'	α9	α13'	α13	α17'	α17
B	B	B	T1'	T1	T5'	T5	T9'	T9	T13'	T13	T17'	T17
C	C	C	α2'	α2	α6'	α6	α10'	α10	α14'	α14	α18'	α18
D	D	D	T2'	T2	T6'	T6	T10'	T10	T14'	T14	T18'	T18
E	E	E	α3'	α3	α7'	α7	α11'	α11	α15'	α15	α19'	α19
F	F	F	T3'	T3	T7'	T7	T11'	T11	T15'	T15	T19'	T19
G	G	G	α4'	α4	α8'	α8	α12'	α12	α16'	α16	α20'	α20
H	H	H	T4'	T4	T8'	T8	T12'	T12	T16'	T16	T20'	T20

Note: A-H, standard wells; α1'-α20', control wells of α-amylase ; α1-α20, sample wells of α-amylase T1'- T20', control wells of (α+β) amylase; T1-T20, sample wells of (α+β) amylase.

▲Operating steps

1. The preparation of standard curve

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, 1.0, 1.2, 1.4 mg/mL.

2. 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 reagent 1 to each tube.

3) Add 150 μL of reagent 2 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.

3. 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 reagent 1 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 reagent 2 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.

4. 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 reagent 1 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 reagent 2 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.

▲Operation table

1. The measurement of standard

	Standard tubes
Standard solution with different concentrations (μL)	75
Reagent 1 (μL)	75
Reagent 2 (μL)	150
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 sample

Reagent	Measurement of α-amylase activity		Measurement of (α+β) amylase activity	
	Control	Sample	Control	Sample
α-Amylase solution (μL)	75	75		
Incubate at 70°C water bath for 15 min and cool down.				
Diluted amylase solution (μL)			75	75
Double distilled water (μL)	75		75	
Reagent 1 (μL)		75		75
Incubate at 40°C water bath for 5 min accurately.				
Reagent 2 (μL)	150	150	150	150
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.				

Note: every sample tube need a control tube.

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.
The standard curve is: y= ax + b.

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.

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

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

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.

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

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

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

Note:

y: $OD_{Standard}$ – OD_{Blank}. (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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 reagent 1 = 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, mgprot/L.

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.97-34.74 U/g tissue	Average inter-assay CV (%)	2.3
Sensitivity	0.97 U/g tissue	Average inter-assay CV (%)	3.2
Average recovery rate (%)	96		

▲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 diluted amylase solution and carry the assay according to the operation table. 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:

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

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

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:

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

$$=14.25 \text{ fresh weight}$$

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