α-Amylase Activity Assay Kit

Catalog No: E-BC-K007-M

Method: Colorimetric method

Specification: 96T (Can detect 40 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 α-amylase activity in animal and plant tissue samples.

▲Background

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. The pancreas and salivary gland make amylase (α-amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria also produce amylase. As diastase, amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833). Specific amylase proteins are designated by different Greek letters. All amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds.

▲Detection principle

The reducing sugar reacts with 3,5-dinitrosalicylic acid under heating conditions to produce a brown-red substance, which is inactivated by the thermolabile nature of β-amylase, and then the enzyme activity of α-amylase is determined.

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

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:

Accurately weigh 0.1 g tissue, add 0.9 mL of double distilled water and mechanical homogenate the sample in ice water bath. Collect the tissue homogenate, stand at room temperature for 15 min and oscillate per 5 min, then centrifuge at 3000 g for 10 min at room temperature, then take the supernatant and add double distilled water to a final volume of 10 mL and it is the prepared sample.

▲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	540 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	S1'	S1	S9'	S9	S17'	S17	S25'	S25	S33'	S33
B	B	B	S2'	S2	S10'	S10	S18'	S18	S26'	S26	S34'	S34
C	C	C	S3'	S3	S11'	S11	S19'	S19	S27'	S27	S35'	S35
D	D	D	S4'	S4	S12'	S12	S20'	S20	S28'	S28	S36'	S36
E	E	E	S5'	S5	S13'	S13	S21'	S21	S29'	S29	S37'	S37
F	F	F	S6'	S6	S14'	S14	S22'	S22	S30'	S30	S38'	S38
G	G	G	S7'	S7	S15'	S15	S23'	S23	S31'	S31	S39'	S39
H	H	H	S8'	S8	S16'	S16	S24'	S24	S32'	S32	S40'	S40

Note: A-H, standard wells; S1'-S40', control wells; S1-S40, sample wells.

▲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 samples

1) Sample tube: Add 75 μL of sample to the corresponding tubes.
   Control tube: Add 75 μL of sample 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.

▲Operation table

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.	

The measurement of sample

	Control tubes	Sample tubes
Sample (μL)	75	75
Incubate at 70°C water bath for 15 min and cool the tubes with running water.		
Double distilled water (μL)	75	
Reagent 1 (μL)		75
Incubate the sample tubes and control tubes at 40°C water bath for 5 min.		
Reagent 2 (μL)	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: $y = ax + b$.

Tissue 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.

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

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.

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

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 sample 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/mL).

▲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 intra-assay CV (%)	3
Sensitivity	0.97 U/g tissue	Average inter-assay CV (%)	3.9
Average recovery rate (%)	98		

▲Example analysis

Take 0.1 g of green pepper, treat the sample according to the sample preparation and carry the assay according to the operation table.

The results are as follows:

standard curve: y= 0.8729x –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 is:

α-Amylase activity (U/g tissue)

=(0.368-0.247+0.0112)÷0.8729×0.15÷5÷0.1×10÷0.075=6.06 U/g tissue