β-Amylase (β-AL) Activity Assay Kit, Microplate Method

Product code: 111988

Product Introduction

Amylases hydrolyze starch and mainly include α-amylase and β-amylase. β-Amylase (EC 3.2.1.2) acts on α-1,4-glycosidic bonds in starch, producing glucose, maltose, maltotriose, dextrin, and other reducing sugars. Reducing sugars reduce 3,5-dinitrosalicylic acid to form a reddish-brown substance.

α-Amylase is not acid-resistant, and β-amylase is not heat-resistant. Based on these characteristics, one enzyme can be inactivated to determine the activity of the other amylase.

Example test sample: sweet potato. OD540 nm readings: α-amylase assay 1.121/1.102, control 0.457/0.439; total amylase assay 0.809/0.807, control 0.182/0.186. Actual readings may vary depending on detection conditions and instrument performance. These data are for reference only.

Product Packing List

Size	Code	Component	Quantity
100T	111988.1	Reagent 1	30 mL
100T	111988.2	Reagent 2	20 mL
100T	111988.3	Standard	5 mg
100T	111988.m	Manual	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent 1	Not specified	Not specified
Reagent 2	Not specified	Not specified
Standard	Not specified	Not specified

Transportation and Storage

Item	Condition
Transportation	Transport with ice packs.
Storage	Store Reagent 1 at 2-8°C protected from light. Store Reagent 2 at room temperature.
Shelf life	180 days

Instructions for Use

1. Preparation of Crude Enzyme Extract

Tissue Samples

1. Weigh 0.1-0.2 g sample. Approximately 0.1 g is recommended.
2. Add 1 mL distilled water and grind to homogenize.

3. Transfer the homogenate to a centrifuge tube and allow it to extract at room temperature for 15 min. Vortex once every 5 min to ensure full extraction.
4. Centrifuge at 3000 g and 25°C for 10 min.
5. Collect the supernatant and bring the volume to 10 mL with distilled water. Mix well. This is the amylase stock solution.
6. Pipette 1 mL amylase stock solution, add 4 mL distilled water, and mix well. This is the diluted amylase solution used to determine total (α + β) amylase activity.

Liquid Samples Such as Serum or Plasma

1. Use the sample directly to detect α-amylase.
2. Pipette 1 mL amylase stock solution, add 4 mL distilled water, and mix well. This is the diluted amylase solution used to determine total (α + β) amylase activity.

2. Solution Preparation

Standard: before use, add 1 mL distilled water to dissolve the standard and prepare a 5 mg/mL standard solution. The prepared standard solution can be stored at 2-8°C for one week.

3. Assay Procedure

1. Preheat the microplate reader for at least 30 min and set the wavelength to 540 nm.
2. Preheat Reagent 1 and Reagent 2 at 40°C for 10 min.
3. Dilute the 5 mg/mL standard solution with distilled water to prepare 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL standard solutions for testing.

No.	Concentration Before Dilution (mg/mL)	Standard Solution Volume (μL)	Distilled Water Volume (μL)	Concentration After Dilution (mg/mL)
1	5	100	900	0.5
2	0.5	200	200	0.25
3	0.25	200	200	0.125
4	0.125	200	200	0.0625
5	0.0625	200	200	0.03125

> In the following experiment, each standard tube requires 75 μL standard solution. Do not measure absorbance directly at this step.

4. Add 75 μL amylase stock solution to one tube and 75 μL diluted amylase solution to another tube. Place both tubes in a boiling water bath for 30 min. These are used as the α-amylase control tube and total amylase control tube, respectively.

4. Operation Table

Component or Step	α-Amylase Control Tube	α-Amylase Assay Tube	Total Amylase Control Tube	Total Amylase Assay Tube	Blank Tube	Standard Tube
Amylase stock solution	75 μL, boiled	75 μL				
Distilled water	75 μL					
Standard solution	75 μL					
70°C water bath	Incubate for approximately 15 min, then quickly place on ice to cool.					
Diluted amylase solution	75 μL, boiled	75 μL				
Reagent 2	75 μL	75 μL	75 μL	75 μL		
40°C incubation	Incubate accurately in a constant-temperature water bath for 10 min.					
Reagent 2	75 μL	75 μL				
Reagent 1	150 μL	150 μL	150 μL	150 μL	150 μL	150 μL
Color development and reading	Mix well, incubate in a 95°C water bath for 10 min, cool, and read absorbance at 540 nm. Record the readings from left to right as A1, A2, A3, A4, A5, and A6.					

Calculate ΔAα = A2 - A1, ΔAtotal = A4 - A3, and ΔAstandard = A6 - A5. Each assay tube requires one control tube. The blank tube

and standard curve only need to be measured once or twice.

Activity Calculation

1. Preparation of the Standard Curve

Use the standard tube concentration (X, mg/mL) and absorbance ΔAstandard (Y, ΔAstandard) to establish the standard curve. Substitute ΔAα (Y, ΔA) into the formula to calculate the sample concentration X1 (mg/mL). Substitute ΔAtotal into the formula to calculate the sample concentration X2 (mg/mL).

2. α-Amylase Activity

Unit definition by sample mass: the amount that catalyzes the production of 1 mg reducing sugar per g tissue per minute is defined as 1 enzyme activity unit.

α-Amylase activity (mg/min/g, fresh weight) = X1 × Vtotal reaction ÷ (W × Vsample ÷ Vtotal sample) ÷ T

Unit definition by protein content: the amount that catalyzes the production of 1 mg reducing sugar per mg tissue protein per minute is defined as 1 enzyme activity unit.

α-Amylase activity (mg/min/mg prot) = X1 × Vtotal reaction ÷ (Vsample × Cpr) ÷ T

For serum, plasma, and other liquid samples, the amount that catalyzes the production of 1 mg reducing sugar per mL sample per minute is defined as 1 enzyme activity unit.

α-Amylase activity (mg/min/mL) = X1 × Vtotal reaction ÷ Vsample ÷ T

3. Total Amylase Activity

Unit definition by sample mass: the amount that catalyzes the production of 1 mg reducing sugar per g tissue per minute is defined as 1 enzyme activity unit.

Total amylase activity (mg/min/g, fresh weight) = 5 × X2 × Vtotal reaction ÷ (W × Vsample ÷ Vtotal sample) ÷ T

Unit definition by protein content: the amount that catalyzes the production of 1 mg reducing sugar per mg tissue protein per minute is defined as 1 enzyme activity unit.

Total amylase activity (mg/min/mg prot) = 5 × X2 × Vtotal reaction ÷ (Vsample × Cpr) ÷ T

For serum, plasma, and other liquid samples, the amount that catalyzes the production of 1 mg reducing sugar per mL sample per minute is defined as 1 enzyme activity unit.

Total amylase activity (mg/min/mL) = 5 × X2 × Vtotal reaction ÷ Vsample ÷ T

4. β-Amylase Activity

β-Amylase activity (mg/min/g, fresh weight) = total amylase activity - α-amylase activity

Calculation Parameters

Symbol	Meaning	Value or Unit
5	Dilution factor of total amylase	5
Vtotal reaction	Total volume of the reaction system	0.15 mL
Vsample	Sample volume added to the reaction system	0.075 mL
Vtotal sample	Total volume of extract	10 mL
Cpr	Sample protein concentration	mg/mL
W	Sample mass	g
T	Reaction time	10 min

Precautions

1. Before formal measurement, select 2-3 samples with large expected differences for preliminary testing. This 100T kit can measure 48 samples.
2. Required instruments and supplies: microplate reader, analytical balance, homogenizer or mortar, refrigerated centrifuge, water bath, adjustable pipettes, 96-well plate, ice, and distilled water.
3. If yellow crystals precipitate from Reagent 1, heat at 60°C to dissolve before use.
4. If precipitation appears in Reagent 2, heat at 70°C to dissolve before use.
5. The linear range of this kit is 0.0078125-1 mg/mL.
6. If the measured absorbance is greater than 1.8, dilute the sample appropriately before measurement. If the absorbance is too low, concentrate the amylase diluent or amylase stock solution.
7. This product is intended for scientific research by professionals only. It must not be used for clinical diagnosis or treatment, must not be used in food or drugs, and must not be stored in ordinary residential premises.
8. For safety and health, wear a lab coat and disposable gloves during operation.

Appendix

For greater accuracy, prepare the standard curve before use. Follow the operation table above. The standard curve formula may be used, or a standard curve may be prepared from the absorbance values of each standard well obtained according to the operation table. Use R2 ≥ 0.99 to obtain the calculation formula for sample calculation.

Visual Reference