β-Amylase(β-AL) Activity Assay Kit

Product Code: 111983

β-Amylase (β-AL) Activity Assay Kit

Product Information

Product Code	111983
Method	Spectrophotometry
Detection Wavelength	540 nm
Kit Size	50T

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 and produces 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 type of amylase can be measured by inactivating the other.

Example test sample: sweet potato. OD540 nm readings: α-amylase assay 1.843/1.879, control 0.858/0.871; total amylase assay 1.406/1.465, control 0.347/0.351. Actual readings may vary depending on the detection instrument and test conditions. These data are for reference only.

Package Contents

Component Code	Component	Quantity
BR5000020.1	Reagent One	50 mL
BR5000020.2	Reagent Two	30 mL
BR5000020.3	Standard	5 mg
BR5000020.m	Instruction Manual	1 copy

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent One	--	--
Reagent Two	--	--
Standard	--	--

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store Reagent I at 2-8°C protected from light. Store Reagent II 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 into 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 dilute 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 to obtain the diluted amylase solution for total (α + β) amylase activity determination.

Serum, Plasma, and Other Liquid Samples

1. Use the amylase stock solution directly for the α-amylase assay.
2. Pipette 1 mL amylase stock solution, add 4 mL distilled water, and mix well to obtain the diluted amylase solution for total (α + β) amylase activity determination.

2. Solution Preparation

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

3. Assay Preparation

1. Preheat the spectrophotometer for at least 30 min. Set the wavelength to 540 nm and zero with distilled water.
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.

Standard Solution Dilution

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	500	500	0.25
3	0.25	500	500	0.125
4	0.125	500	500	0.0625
5	0.0625	500	500	0.03125

> In the assay below, each standard tube requires 250 μL standard solution. Do not measure absorbance directly at this dilution step.

4. Control Preparation

Prepare two tubes. Add 250 μL amylase stock solution to one tube and 250 μL amylase dilution solution to the other tube. Boil both tubes in a water bath for 30 min. Use them as the α-amylase control tube and total amylase control tube, respectively.

5. Assay Procedure

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 (μL)	250, boiled	250				
Distilled water (μL)	250					
Standard solution (μL)	250					
Water bath	70°C for approximately 15 min, then cool quickly on ice	70°C for approximately 15 min, then cool quickly on ice				

Amylase dilution solution (μL)	250, boiled	250				
Reagent II (μL)	250	250	250	250		
Incubation	40°C constant-temperature water bath for exactly 10 min	40°C constant-temperature water bath for exactly 10 min	40°C constant-temperature water bath for exactly 10 min	40°C constant-temperature water bath for exactly 10 min		
Reagent II (μL)	250	250				
Reagent I (μL)	500	500	500	500	500	500
Color development and reading	Mix well, heat in a 95°C water bath for 10 min, cool, and read absorbance at 540 nm.					

Record the absorbance values from left to right as A1, A2, A3, A4, A5, and A6.

Calculate: ΔAα = A2 - A1; ΔAtotal = A4 - A3; 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. 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 standard curve formula to calculate sample concentration X1 (mg/mL). Substitute ΔAtotal into the formula to calculate sample concentration X2 (mg/mL).

2. α-Amylase Activity

Calculated by sample mass: each g of tissue catalyzing the production of 1 mg reducing sugar 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

Calculated by protein content: each mg of tissue protein catalyzing the production of 1 mg reducing sugar is defined as 1 enzyme activity unit.

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

For serum, plasma, and other liquid samples: each mL serum or plasma catalyzing the production of 1 mg reducing sugar per minute is defined as 1 enzyme activity unit.

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

3. Total Amylase Activity

Calculated by sample mass: each g of tissue catalyzing the production of 1 mg reducing sugar per minute is defined as 1 enzyme activity unit.

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

Calculated by protein content: each mg of tissue protein catalyzing the production of 1 mg reducing sugar per minute is defined as 1 enzyme activity unit.

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

For serum, plasma, and other liquid samples: each mL serum or plasma catalyzing the production of 1 mg reducing sugar per minute is

defined as 1 enzyme activity unit.

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

4. β-Amylase Activity

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

Formula Parameters

Symbol	Description
5	Total amylase dilution factor
Vreaction total	Total volume of the reaction system, 0.5 mL
Vsample	Sample volume added to the reaction system, 0.25 mL
Vsample total	Total volume of extract, 10 mL
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
T	Reaction time, 10 min

Notes

1. Before formal measurement, select 2-3 samples with large expected differences for preliminary testing. This 50T reagent kit can test 24 samples.
2. Required instruments and supplies: visible spectrophotometer, analytical balance, homogenizer or mortar, refrigerated centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, ice, and distilled water.
3. If yellow crystals precipitate from Reagent I, heat at 60°C to dissolve before use.
4. If Reagent II shows precipitate, heat at 70°C to dissolve before use.
5. The linear range of this kit is 0.03125-0.5 mg/mL.
6. If the measured absorbance value is greater than 2, dilute the sample appropriately before measurement. If the absorbance value is too low, concentrate the amylase diluent or the original amylase solution.
7. This product is 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 residences.
8. For safety and health, wear a lab coat and disposable gloves during operation.

Appendix

For greater accuracy, prepare a standard curve for each experiment. Use the procedure table above to obtain absorbance values for each standard tube, plot the standard curve with $R^2 \geq 0.99$, and use the resulting calculation formula for sample calculation.

Visual Reference