α-Amylase (α-AL) Activity Assay Kit - Micro Method

Product Information

Product Code: 111969

Starch hydrolases include α-amylase and β-amylase. α-Amylase (α-AL, EC 3.2.1.1) randomly catalyzes the hydrolysis of α-1,4-glycosidic bonds in starch, producing glucose, maltose, maltotriose, dextrin, and other reducing sugars while reducing starch viscosity. For this reason, it is also called a liquefying enzyme.

Starch hydrolase catalyzes starch hydrolysis to produce reducing sugars. These reducing sugars reduce 3,5-dinitrosalicylic acid to form a reddish-brown substance with an absorption peak at 540 nm. Amylase activity is calculated by measuring the rate of increase in absorbance at 540 nm.

α-Amylase is heat-resistant, while β-amylase can be inactivated at 70°C for 15 min. After the crude enzyme solution is treated at 70°C for 15 min, only α-amylase can catalyze starch hydrolysis.

Package Contents

Product Code	Component	Quantity	Storage
111969.1	Reagent I	15 mL	2-8°C, protected from light
111969.2	Reagent II	7.5 mL	2-8°C
111969.m	Instruction Manual	1 copy	-

Quality and Safety Information

Raw Material or Package Name	Quality Standard	Main Toxicity
Reagent I	-	-
Reagent II	-	-

Transportation and Storage

Transportation: This product is transported with ice packs.

Storage: Store according to the instructions. Shelf life: 180 days.

Instructions for Use

1. Preparation of Crude Enzyme Extract

Tissue samples: Weigh approximately 0.1 g of sample. Add 1 mL distilled water and homogenize. After homogenization, leave at room temperature for 15 min for extraction, vortexing once every 5 min to ensure sufficient extraction. Centrifuge at 3000 g at room temperature for 10 min. Collect the supernatant, bring to 10 mL with distilled water, and mix well to obtain the amylase stock solution.

Serum or plasma samples: Test directly.

2. Assay Procedure

1. Preheat the microplate reader for more than 30 min. Set the wavelength to 540 nm and blank with distilled water.
2. Prepare EP tubes and operate according to the table below.

Component or Step	Control Tube	Assay Tube
Amylase stock solution	75 μL	75 μL
Heat treatment	Water bath at 70°C for 15 min, then cool under running water.	
Distilled water	75 μL	-
Reagent II	-	75 μL
Incubation	Incubate accurately in a 40°C constant-temperature water bath for 5 min.	
Reagent I	150 μL	150 μL
Color development and reading	Mix well. Heat in a 95°C water bath for 5 min, cool, transfer 200 μL into a 96-well plate, and read the absorbance of the control tube and assay tube at 540 nm.	

Calculate ΔA as follows:

$\Delta A = A_{assay} - A_{control}$

α-Amylase Activity Calculation

1. Calculation Using a Micro Quartz Cuvette

The regression curve measured under standard conditions is:

y = 3.7215x - 0.1778

where x is the standard concentration (mg/mL), and y is the absorbance value.

1.1 Calculation by Sample Mass

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

α-Amylase activity (mg/min/g fresh weight) = $[(\Delta A + 0.1778) \div 3.7215 \times V_{sample}] \div (W \times V_{sample} \div V_{total\ sample}) \div T = 0.5374 \times (\Delta A + 0.1778) \div W$

1.2 Calculation by Protein Content

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

α-Amylase activity (mg/min/mg prot) = $[(\Delta A + 0.1778) \div 3.7215 \times V_{sample}] \div (V_{sample} \times Cpr) \div T = 0.05374 \times (\Delta A + 0.1778) \div Cpr$

1.3 Calculation for Serum or Plasma Samples

Unit definition: The amount of enzyme in each mL of serum or plasma that catalyzes the production of 1 mg reducing sugar per minute is defined as 1 enzyme activity unit.

α-Amylase activity (mg/min/mL) = $[(\Delta A + 0.1778) \div 3.7215 \times V_{sample}] \div V_{sample} \div T = 0.05374 \times (\Delta A + 0.1778)$

Parameters for Micro Quartz Cuvette Calculation

- V_{sample}: sample volume added to the reaction system, 0.075 mL
- $V_{total\ sample}$: total extract volume, 10 mL
- Cpr: sample protein concentration, mg/mL
- W: sample mass, g
- T: reaction time, 5 min

2. Calculation Using a 96-Well Plate

The regression curve measured under standard conditions is:

y = 2.481x - 0.1778

where x is the standard concentration (mg/mL), and y is the absorbance value.

2.1 Calculation by Sample Mass

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

α-Amylase activity (mg/min/g fresh weight) = $[(\Delta A + 0.1778) \div 2.481 \times V_{sample}] \div (W \times V_{sample} \div V_{total\ sample}) \div T = 0.806 \times (\Delta A + 0.1778) \div W$

2.2 Calculation by Protein Content

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

α-Amylase activity (mg/min/mg prot) = $[(\Delta A + 0.1778) \div 2.481 \times V_{sample}] \div (V_{sample} \times Cpr) \div T = 0.0806 \times (\Delta A + 0.1778) \div Cpr$

2.3 Calculation for Serum or Plasma Samples

Unit definition: The amount of enzyme in each mL of serum or plasma that catalyzes the production of 1 mg reducing sugar per minute is defined as 1 enzyme activity unit.

α-Amylase activity (mg/min/mL) = $[(\Delta A + 0.1778) \div 2.481 \times V_{sample}] \div V_{sample} \div T = 0.0806 \times (\Delta A + 0.1778)$

Parameters for 96-Well Plate Calculation

- V_{sample}: sample volume added to the reaction system, 0.075 mL
- $V_{total\ sample}$: total volume of extract, 10 mL
- Cpr: sample protein concentration, mg/mL
- W: sample mass, g
- T: reaction time, 5 min

Precautions

1. If yellow crystals precipitate in Reagent I, heat at 90°C to dissolve before use.
2. If precipitate forms in Reagent II, heat at 70°C to dissolve before use.
3. This 100T product can test 48 samples.
4. Instruments and reagents to be prepared by the user: microplate reader or spectrophotometer, 96-well plate or 1 mL glass cuvette, mortar or homogenizer, adjustable pipette, benchtop centrifuge, constant-temperature water bath, and distilled water.
5. 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.
6. For your safety and health, wear a lab coat and disposable gloves during operation.