Technical Manual

Alpha-Amylase and Beta-amylase Activity Assay Kit

- Catalogue Code: MAES0019
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.01-0.37 U/mL

Sensitivity:

0.008 U/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Amylase is a general term for hydrolase and glycogen enzymes, which generally acts on soluble starch, amylose, glycogen, etc., and is widely present in animals, plants and microorganisms. It is one of the most important enzymes in all industrial enzymes.

3. Intended Use

This kit can measure α- and β-amylase activity in serum, plasma, saliva, tissue samples.

4. Detection Principle

The reducing sugar reacts with 3,5-dinitrosalicylic acid under heating conditions to produce a brown-red substance. Amylase activity can be calculated by measuring the OD value at 540 nm.

5. Kit components & storage

Item	Specification	Storage
Substrate	10 mL×1 vial	2-8°C, 6 months
Chromogenic Agent	20 mL×1 vial	2-8°C, 6 months, away from direct sunlight
Standard (10 mg/mL)	1.5 mL×1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (540 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

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 greater than 0.800, sample should be diluted appropriately.

7. Reagent Preparation

1. Bring all reagents to room temperature before use. Before the experiment, preheat substrate and chromogenic agent at 40°C for 10 min.

2. If there is precipitation in substrate, please use it after heating and dissolving at 70°C.

3. If there is yellow precipitation in chromogenic agent, please use it after heating and dissolving at 70°C.

8. Sample Preparation

1. **Serum (Plasma):** Detect the sample directly.

2. **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.

3. **Saliva sample**: Use a sterile container to collect saliva samples. Remove particulates by centrifugation for 10 min at 3000 g. It is recommended to use fresh samples.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01-0.37 U/mL).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Human plasma	10-15
Human serum	10-15
Human saliva	15-25
Mouse serum	15-25
Rat serum	15-25
1% Corn grain tissue homogenate	2-5

Note: The diluent is double distilled water.

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 540 nm

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.

9. Operation Steps

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.

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 substrate to each tube.

3. Add 150 μL of chromogenic agent 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.

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. **Sample tube:** Add 75 μL of substrate 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 chromogenic agent 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

Measurement of Standard	Standard tubes
Standard solution with different concentrations (μL)	75
Substrate (μL)	75
Chromogenic agent (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.	

Measurement of Samples	Control tubes	Sample tubes
Sample (μL)	75	75
Double distilled water (μL)	75	
Substrate (μL)		75
Incubate the sample tubes and control tubes at 40℃ water bath for 5 min.		
Chromogenic agent (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.		

10. Calculations

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. **Serum (plasma) and other liquid sample:**
   Definition: The production of 1 mg reducing sugar catalyzed by 1 mL of sample per minute that is defined as an enzyme activity unit.

$$(\alpha+\beta)\ \text{Amylase activity (U/mL)} = (\Delta A - b) \div a \times V_3 \div t \div V_2 \times f$$

2. **Tissue sample:**
   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.

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

   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.

$$(\alpha+\beta)\ \text{Amylase activity (U/g fresh weight)} = (\Delta A - b) \div a \times V_3 \div t \div w \times \frac{V_1}{V_2} \times f$$

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 substrate = 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).

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11. Performance Characteristics

Detection Range	0.01-0.37 U/mL
Sensitivity	0.008 U/mL
Average recovery rate (%)	97
Average inter-assay CV (%)	3.6
Average intra-assay CV (%)	2.8

Analysis

Take 10 μL of rat serum, dilute with double distilled water 25 times and complete 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 0.299, the average OD value of the control is 0.162, and the calculation result is:

$$(\alpha+\beta)\textbf{Amylase activity}\left(\frac{U}{mL}\right) = (0.299 - 0.162 + 0.0112) \div (0.8729 \times 0.15) \div 5 \div (0.075 \times 25) = 1.70\ U/mL$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching 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.

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

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