TECHNICAL MANUAL

alpha-Amylase Activity Assay Kit

- **SKU CODE:** MAES0020
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. Detection principle

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

3. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Substrate	5 mL x 1 vial	10 mL x 1 vial	50 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Chromogenic Agent	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8 °C, 12 months, shading light
Reagent 3	10 mg/mL Standard	1.5 mL x 1 vial	1.5 mL x 1 vial	7.5 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces		

4. Materials prepared by users

Instruments: Test tubes, Vortex Mixer, Centrifuge, Water bath, Microplate reader (535-540 nm, optimum wavelength: 540 nm)

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. If there is precipitation in substrate and chromogenic agent, heat at 70-80 °C in a water bath until dissolved. Cool down to 40 °C with fresh water before use.

3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 mg/mL standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/mL. Reference dilution table: Item: ①②③④⑤⑥⑦⑧ Concentration (mg/mL): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 10 mg/mL standard (μL): 0, 20, 40, 60, 80, 100, 120, 140 Double distilled water (μL): 1000, 980, 960, 940, 920, 900, 880, 860

6. Sample preparation

1. **Sample preparation:** Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for one month. Saliva sample: Gargle with clear water, collect saliva 30 min later, centrifuge at 10,000 x g for 10 min at 4 °C. Take the supernatant and preserve it on ice for detection. Tissue sample: 1) Weigh 0.1 g sample, add 0.9 mL of distilled water and homogenize with a homogenizer in an ice water bath, then transfer to an EP tube. Incubate at room temperature for 15 min and oscillate every 5 min. 2) Centrifuge at 3,000 x g at room temperature for 10 min, take the supernatant and add double distilled water to a final volume of 10 mL. This is the prepared sample.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 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. For dilution of other sample types, perform a pretest to confirm the dilution factor.

7. The key points of the assay

1. For measuring the OD value, if there is precipitation, centrifuge at 4,000 x 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.

8. Operating steps

1. **Measurement of standard:** 1) Take 1.5 mL EP tubes and number them from A to H in duplicate. 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.

2. **Measurement of sample:** 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 substrate 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 chromogenic agent 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.

9. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) and saliva sample:

Definition: The production of 1 mg reducing sugar catalyzed by 1 mL of serum/saliva per minute is defined as one enzyme activity unit.

α-amylase activity (U/mL) = (ΔA - b) ÷ a × V_3 ÷ t ÷ V_2 × f

2. 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 is defined as one enzyme activity unit.

α-amylase activity (U/mgprot) = (ΔA - b) ÷ a × V_3 ÷ t ÷ V_2 × f ÷ 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 is defined as one enzyme activity unit.

α-amylase activity (U/g tissue) = (ΔA - b) ÷ a × V_3 ÷ t ÷ w × V_1/V_2 × f

[Note]

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 (volume of sample + 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).

10. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	0.02	0.15	0.30
%CV	3.3	2.8	2.9

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	0.02	0.15	0.30
%CV	4.1	4.0	3.6

Recovery

Three samples of high, middle and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Recovery

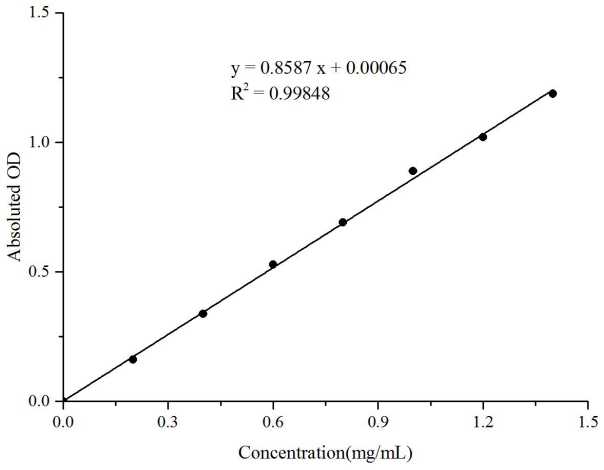
	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.25	0.7	1.1
Observed Conc. (mg/mL)	0.3	0.7	1.1
Recovery rate (%)	100	95	99

Sensitivity

The analytical sensitivity of the assay is 0.0097 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mg/mL)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.139	0.140	0.140	0.000
0.2	0.310	0.292	0.301	0.161
0.4	0.465	0.489	0.477	0.338
0.6	0.668	0.669	0.668	0.529
0.8	0.839	0.821	0.830	0.690
1.0	1.018	1.040	1.029	0.889
1.2	1.142	1.176	1.159	1.019
1.4	1.339	1.317	1.328	1.188

11. Appendix II Example Analysis

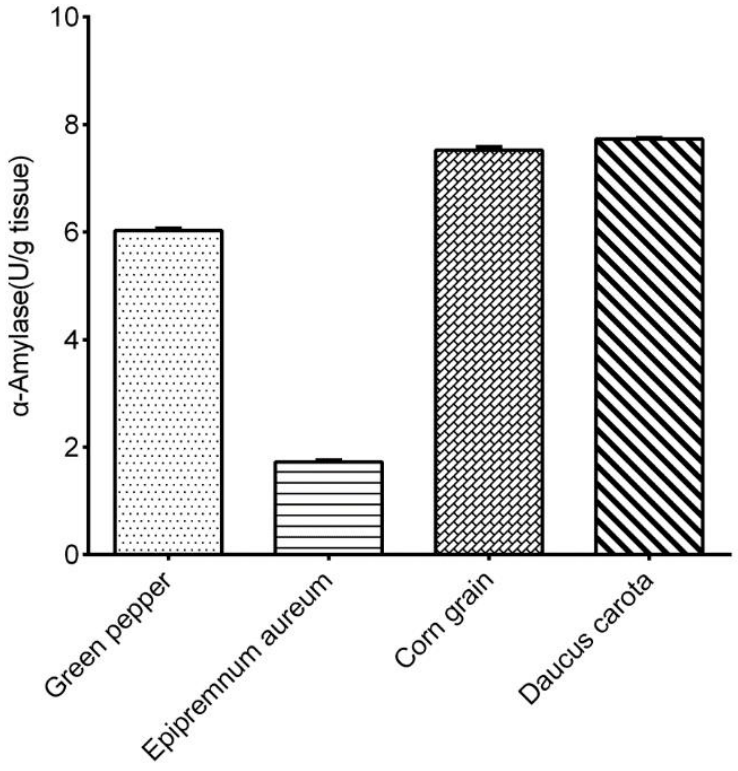
Example analysis:

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

Detect 1% green pepper tissue homogenate, 1% epipremnum aureum tissue homogenate, 1% corn grain tissue homogenate and 1% daucus carota tissue homogenate according to the protocol. The result is as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.