TECHNICAL MANUAL

alpha-Amylase and beta-amylase Activity Assay Kit

- **SKU CODE:** MAES0019
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. 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.

3. Kit components & storage

Item	Component	Size 1(96 T)	Size 2(500Assays)	Storage
Reagent 1	Substrate	10 mL x 1 vial	50 mL x 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent	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	7.5 mL x 1 vial	2-8°C, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	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 it at 70-80°C in water bath until dissolved. Cool down to 40°C with fresh water before use.
3. **The 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 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.

6. Sample preparation

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 a month.

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 saliva samples.

Tissue sample:

1. Weigh 0.1 g sample, add 0.9 mL of distilled water and homogenize with a homogenizer in ice water bath, then transfer to the EP tube, incubate at room temperature for 15 min and oscillate every 5 min.

2. Centrifuge at 3000×g at room temperature for 10 min, take the supernatant and add double distilled water to the final volume of 10 mL, mix fully and it is the α-amylase solution.

Dilution of sample:

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

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. For the dilution of other sample types, please do 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 4000 g for 5 min at room temperature and take the supernatant for determination.
2. When the absolute OD value is more than 0.800, it is recommended to dilute the sample appropriately.

8. Operating steps

The measurement of standard

① Take 1.5 mL EP tube and number the tubes 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. The 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 Sample tube: add 75 μL of substrate to the corresponding tubes.

Control tube: add 75 μL of double distilled water to the 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.

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 by using absolute OD value of standard and correspondent 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 other saliva 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.

(α+β) 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 that is defined as an enzyme activity unit.

(α+β) 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 that is defined as an enzyme activity unit.

(α+β) Amylase activity (U/g fresh weight) = (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 (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).

10. Appendix I Performance Characteristics

Parameter:

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)	0.08	1.40	0.25
%CV	3.2	2.4	2.8

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)	0.08	1.40	0.25
%CV	3.0	4.0	3.8

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 97%.

Recovery

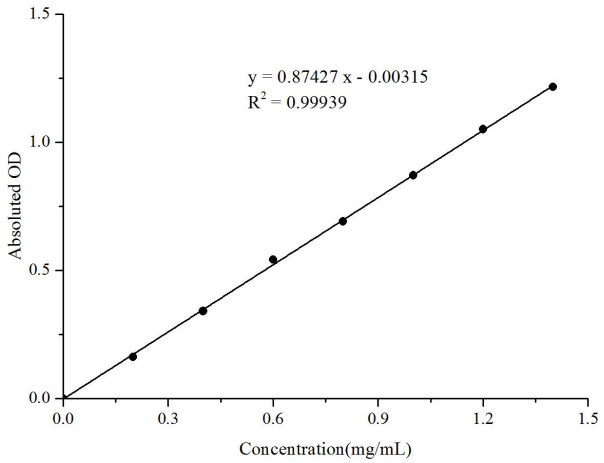
	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/mL)	0.3	0.7	1.1
Observed Conc. (mg/mL)	0.3	0.7	1.1
Recovery rate (%)	99	95	97

Sensitivity

The analytical sensitivity of the assay is 0.008 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 as below for reference only:



Standard curve data

Concentration (mg/mL)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.144	0.144	0.144	0.000
0.2	0.310	0.301	0.305	0.161
0.4	0.483	0.486	0.484	0.341
0.6	0.688	0.684	0.686	0.542
0.8	0.829	0.840	0.834	0.691
1.0	1.016	1.013	1.015	0.871
1.2	1.224	1.164	1.194	1.050
1.4	1.361	1.356	1.359	1.215

11. Appendix II Example Analysis

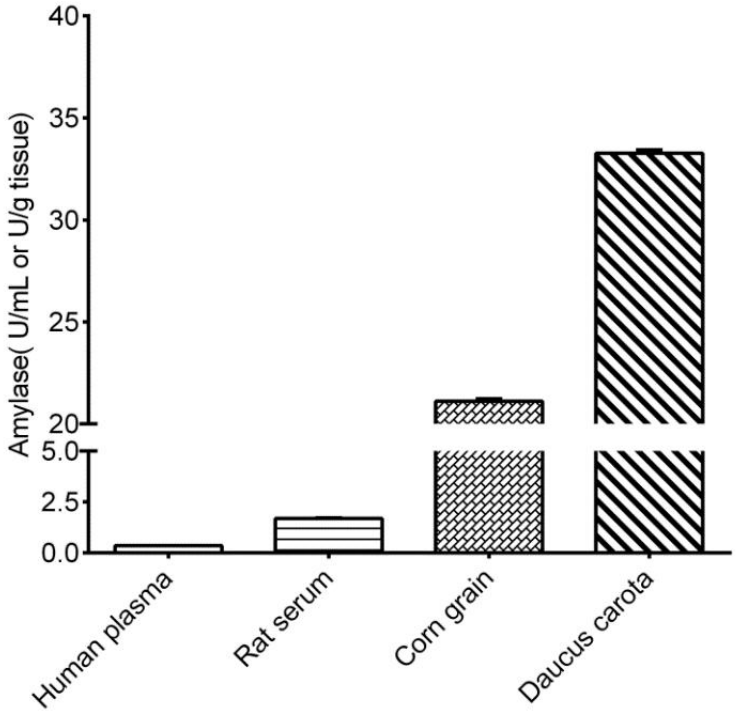
Example analysis:

For rat serum, take 10 μL of rat serum, dilute with double distilled water for 25 times and carry the assay according to the operation steps. 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:

(α+β) Amylase activity (U/mL) =(0.299 - 0.162 + 0.0112) ÷ 0.8729 × 0.15 ÷ 5 ÷ 0.075 × 25 = 1.70 U/mL

Detect human plasma (dilute for 5 times), rat serum (dilute for 25 times), 1% corn grain tissue homogenate (dilute for 5 times) and 1% daucus carota tissue homogenate (dilute for 5 times) 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.