TECHNICAL MANUAL

Maltase Activity Assay Kit

- **SKU CODE:** MAES0053
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure optical density

2. Intended use

This kit can be used to measure maltase activity in animal tissue samples.

3. Detection principle

Maltase catalyzes the corresponding substrate to produce monosaccharide. Monosaccharide produces hydrogen peroxide under the action of oxidase. Hydrogen peroxide reacts with chromogenic agent to form a red product. The activity of maltase can be calculated by detecting the optical density with a spectrophotometer at 505 nm.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Substrate	Powder x 1 vial	2-8 °C, 12 months
Reagent 2	Buffer Solution	8 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Stop Solution	3.5 mL x 1 vial	2-8 °C, 12 months
Reagent 4	Chromogenic Agent A	13 mL x 1 vial	2-8 °C, 12 months, protect from light
Reagent 5	Chromogenic Agent B	13 mL x 1 vial	2-8 °C, 12 months, protect from light
Reagent 6	50 mmol/L Glucose Standard Solution	1 mL x 1 vial	2-8 °C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Micropipettor, Vortex mixer, Centrifuge, Water bath, Incubator, Microplate reader (505 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate other reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 6 mL of buffer solution, mix well to dissolve. Store at 2-8 °C for up to one month.
3. **Preparation of chromogenic reagent:** For each well, prepare 200 µL of chromogenic reagent by mixing 100 µL of chromogenic agent A and 100 µL of chromogenic agent B. The chromogenic reagent should be prepared fresh immediately before use.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 50 mmol/L glucose standard solution with double distilled water to create a serial dilution. The recommended dilution gradient is as follows: 0, 2.5, 5, 10, 15, 20, 25, 30 mmol/L.

7. Sample preparation

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

- 10% Rat heart tissue homogenate: 1

- 10% Rat liver tissue homogenate: 1

- 10% Rat spleen tissue homogenate: 1
- 10% Mouse intestinal tissue homogenate: 1
- 10% Mouse kidney tissue homogenate: 1
- 10% Mouse brain tissue homogenate: 1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. If the OD value of sample tube is more than 1, please dilute the sample and test again.
2. If the maltase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (MAES0177).
3. Accurate operation is required when adding liquid to microplate and prevent the formation of bubbles when adding the liquid to the microplate.

9. Operating steps

For standard curve:

1. Take 1.5 mL EP tubes and number the tubes from A to H in duplicate, add 25 μL of standard solution with different concentrations to the corresponding tubes.

2. Add 50 μL of substrate working solution to each tube.

3. Add 25 μL of stop solution to each tube.

4. Mix well with a vortex mixer and take 8 μL of supernatant to the corresponding wells in microplate.

5. Add 200 μL of chromogenic agent to each well.

6. Mix fully for 5 s with microplate reader, incubate at 37 °C for 15 min and measure the OD value of each well at 505 nm.

For samples:

1. Control tube: add 50 μL of substrate working solution to the corresponding 1.5 mL EP tubes.

Sample tube: add 25 μL of sample and 50 μL of substrate working solution to the corresponding 1.5 mL EP tubes.

2. Mix well with a vortex mixer and react at 37 °C for 20 min.

3. Add 25 μL of stop solution to each tube.

4. Control tube: add 25 μL of sample to the corresponding 1.5 mL EP tubes.

5. Mix well with a vortex mixer and centrifuge at 3,000 xg for 10 min.

6. Take 8 μL of the supernatant to corresponding wells in microplate.

7. Add 200 μL of chromogenic agent to each well.

8. Mix well for 5 s with microplate reader, incubate at 37 °C for 15 min and measure the OD value of each well at 505 nm.

10. Calculation

The standard curve:

1. Average the duplicate readings 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 corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Definition: The amount of 1 nmol of maltose hydrolyzed by 1 mg of tissue protein per minute at 37 °C and pH 6.0 is defined as 1 unit.

Maltase activity (U/mg_{prot}) = (ΔA_505 - b) ÷ a ÷ 2* ÷ t ÷ C_{pr} × 1000**

[Note]

ΔA_505: $OD_{sample} - OD_{control}$.

2*: A maltose molecule can be decomposed into two glucose molecules.

t: Reaction time, 20 min.

1000**: 1 mmol/L = 1000 nmol/mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mg_{prot}/mL

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three mouse intestinal tissue 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)	16.50	247.00	584.00
%CV	3.3	2.6	2.2

Inter-assay Precision

Three mouse intestinal tissue 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)	16.50	247.00	584.00
%CV	6.1	5.6	5.4

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 103%.

Recovery

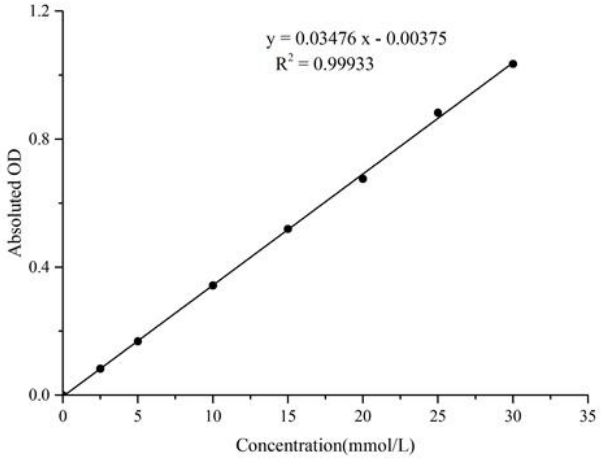
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	4.5	13	24
Observed Conc. (mmol/L)	4.5	13.3	25.4
Recovery rate (%)	101	102	106

Sensitivity

The analytical sensitivity of the assay is 6.32 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

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 (mmol/L)	Average OD	Absolute OD
0	0.154	0
2.5	0.236	0.082
5	0.322	0.168
10	0.496	0.342
15	0.672	0.518
20	0.829	0.675
25	1.036	0.882
30	1.188	1.034

12. Appendix II Example Analysis

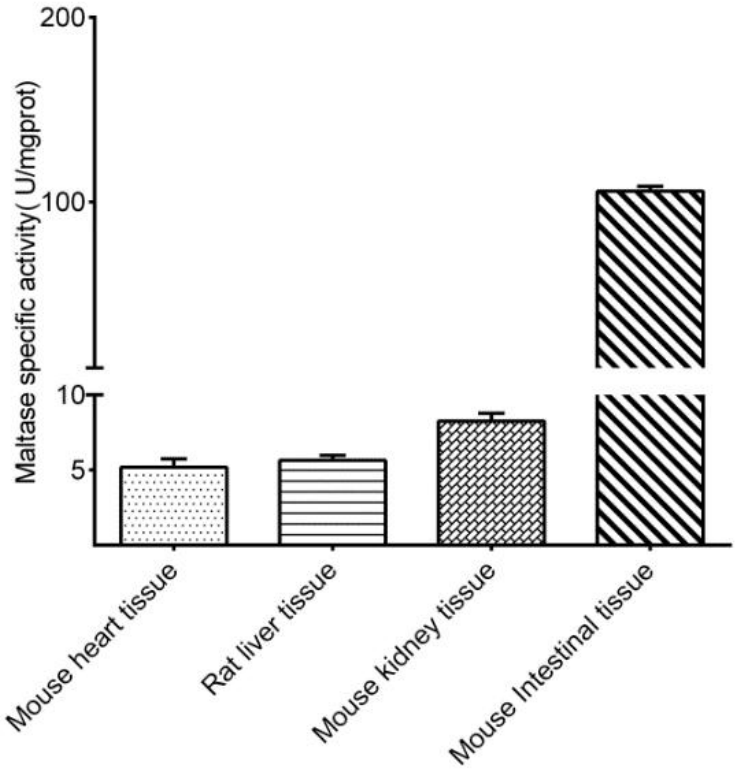
Example analysis:

For mouse intestinal tissue, take 10% fresh mouse intestinal tissue homogenate and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0356x – 0.0009, the average OD value of the sample is 0.900, the average OD value of the control is 0.162, the concentration of protein in sample is 4.56 mg_{prot}/mL, and the calculation result is:

Maltase activity (U/mg_{prot}) = (0.900 – 0.162 + 0.0009) ÷ 0.0356 ÷ 2 ÷ 20 ÷ 4.56 × 1000 = 113.79 U/mg_{prot}

Detect 10% mouse heart tissue homogenate (the concentration of protein is 5.17 mg_{prot}/mL), 10% rat liver tissue homogenate (the concentration of protein is 10.91 mg_{prot}/mL), 10% mouse kidney tissue homogenate (the concentration of protein is 7.96 mg_{prot}/mL) and 10% mouse intestinal tissue homogenate (the concentration of protein is 4.56 mg_{prot}/mL), according to the protocol, the result is as follows:



13. 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.