（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K041-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader (505 nm)

Detection range：6.32-750 U/mL

Elabscience® Maltase Activity Assay Kit

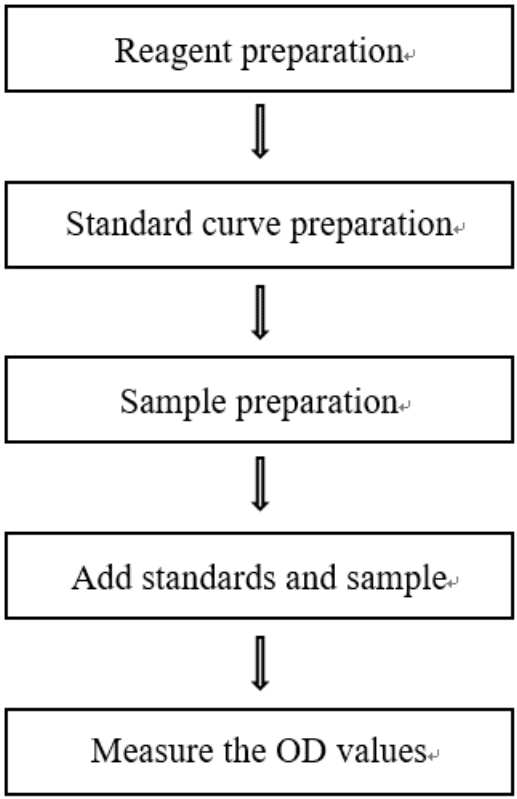
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

Detection principle

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

Kit components & storage

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of substrate working solution:

Dissolve one vial of substrate with 6 mL of buffer solution, mix well to dissolve. Store at 2-8 ℃ for a month.

③ The preparation of chromogenic reagent:

For each well, prepare 200 μL of chromogenic reagent (mix well 100 μL of chromogenic agent A and 100 μL of chromogenic agent B). The chromogenic reagent should be prepared on spot.

④ The 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 diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 2.5, 5, 10, 15, 20, 25, 30 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**2.5**	**5**	**10**	**15**	**20**	**25**	**30**
50 mmol/L glucose standard (μL)	0	20	40	80	120	160	200	240
Double distilled water (μL)	400	380	360	320	280	240	200	160

Sample preparation

① 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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
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 do pretest to confirm the dilution factor

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 lactase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

③ Accurate operation is required when adding liquid to microplate and prevent the formulation of bubbles when adding the liquid to the microplate.

Operating steps

For standard curve:

① Take 1.5 mL EP tube and number the tubes from A to H in duplication, 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℃ 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℃ 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 3000 g 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℃ for 15 min and measure the OD value of each well at 505 nm.

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 absoluted OD value.

3. Plot the standard curve by using absoluted 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:

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

$$\text{Maltase activity (U/mgprot)} = (\Delta A505 - b) \div a \div 2^{*} \div t \div C_{pr} \times 1000^{**}$$

[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, mgprot/mL

.

Appendix I Performance Characteristics

1. 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).

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 were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	16.50	247.00	584.00
%CV	6.1	5.6	5.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	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

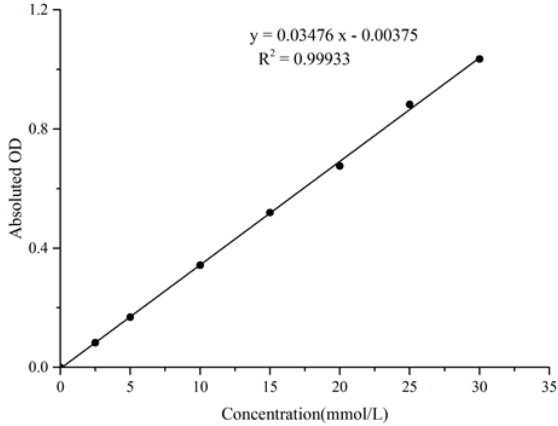
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

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



Appendix II Example Analysis

Example analysis：

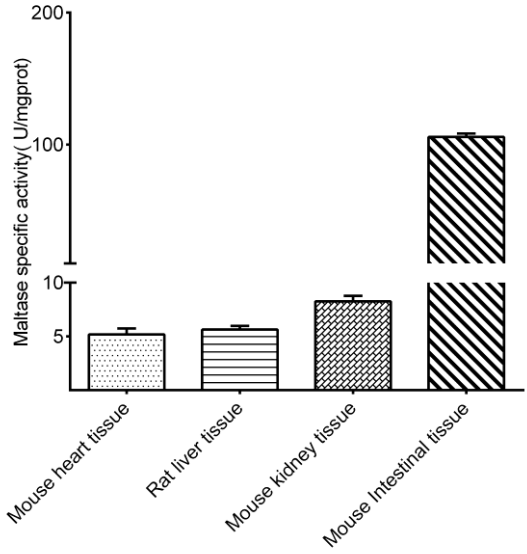
For mouse intestinal tissue, take 10% fresh mouse intestinal tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0356 x – 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 mgprot/mL, and the calculation result is:

Maltase activity (U/mgprot)

$= (0.900 - 0.162 + 0.0009) \div 0.0356 \div 2 \div 20 \div 4.56 \times 1000 = 113.79$ U/mgprot

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



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.