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

Maltase Assay Kit

Catalog No.: BC00185

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Maltase Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell
Assay Type	Quantitative
Detection	Spectrophotometer, Microplate reader (505 nm)

Principle

Maltase acts on the corresponding substrate to produce monosaccharides.

Under the action of oxidase, the monosaccharides generate hydrogen peroxide, which binds with chromogenic reagent to form red products. The optical density value is measured by a spectrophotometer to determine maltase activity.

Components

No.	Components	Size	Notes
Reagent 1	Powder	1 vial	Store at 2-8°C. Before use, add 10 mL of diluent to powder, dissolve thoroughly to prepare the Substrate Solution, and set aside for later use.
	Diluent	10 mL	
Reagent 2	Stop Solution	5 mL	Store at 2-8°C
Reagent 3	Liquid	50 mL	Store at 2-8°C
Reagent 4	Glucose Standard Solution	5.55 mmol/L	Store at 2-8°C. Before use, dilute with distilled water at a volume ratio of 1:2 to prepare 1.85 mmol/L glucose standard solution.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The kit has a validity period of 6 months.

Preparation

Visible spectrophotometer with adjustable wavelength of 505 nm, 1 cm optical path cuvettes, vortex mixer, centrifuge, 37°C water bath, stopwatch, distilled water, normal saline, chloroform (for high-fat samples), and protein assay reagents (for tissue or cell samples, available from our company).

- **Sample handling**

1. Liquid samples: Detect directly. If the result exceeds the linear range, dilute with normal saline before detection.
2. Tissue samples: Accurately weigh the tissue sample. Add 9 volumes of homogenate medium at a mass-to-volume ratio of 1:9 (g:mL). Perform mechanical homogenization in an ice-water bath, then centrifuge at 2500 rpm for 10 minutes. Collect the supernatant for subsequent testing.
   Note: 0.1 mol/L phosphate buffer (pH 7.4) or 0.9% normal saline can be used as the homogenate medium.
3. Cell samples:
   Cell collection: Take the prepared cell suspension and centrifuge at 1000 rpm for 10 minutes. Discard the supernatant and retain the cell pellet. Rinse the pellet 1–2 times with isotonic buffer (recommended: 0.1 mol/L phosphate buffer, pH 7–7.4). Centrifuge again at 1000 rpm for 10 minutes and discard the supernatant to preserve the cell pellet.
   Cell disruption: Add 0.2–0.3 mL of homogenate medium (recommended: 0.1 mol/L phosphate buffer or normal saline, pH 7–7.4) for homogenization. Perform ultrasonic disruption in an ice-water bath (power: 300 W, 3–5 seconds per cycle, 30-second interval, repeat 3–5 times) or manual homogenization. The prepared homogenate can be tested directly without centrifugation. Alternatively, lyse cells with lysis buffer (1%–2% Triton X-100 is recommended) for 30–40 minutes. The lysate can be tested directly without centrifugation.
   Note: The cell density is recommended to be higher than 1×10^6 cells/mL. The disrupted sample can be observed under a microscope to verify complete cell lysis.

Operation process

1. Enzymatic Reaction:

	Assay tube	Control tube
Sample (μL)	25	
Substrate Solution (μL)	50	50
Mix well and incubate at 37 °C for 20 minutes.		
Stop Solution (μL)	25	25
Sample (μL)		25
Mix thoroughly, centrifuge at 4000 rpm for 10 minutes, and collect the supernatant for color reaction.		

2. Color Reaction (Microplate Reader)：

	Blank well	Standard well	Assay well	Control well
Distilled water (μL)	8			
1.85 mmol/L Glucose Standard Solution (μL)		8		
Supernatant of enzymatic reaction (μL)			8	8
Reagent 3 (μL)	200	200	200	200
Gently shake the microplate, incubate at 37 °C for 15 minutes, and read the absorbance using a microplate reader at 505 nm.				

Color Reaction (Spectrophotometer)：

	Blank tube	Standard tube	Assay tube	Control tube
Distilled water (μL)	40			
1.85 mmol/L Glucose Standard Solution (μL)		40		

Supernatant of enzymatic reaction (μL)			40	40
Reagent 3 (μL)	1000	1000	1000	1000
Mix well, incubate at 37 °C for 15 minutes, zero the spectrophotometer with distilled water, and perform colorimetry at 505 nm.				

Notes: Before the formal experiment, select individual samples (one from the normal group and one from the model group) for a concentration gradient pre-experiment (dilution factors of 5-fold, 10-fold, 20-fold or others are optional), so as to determine the optimal sample concentration. At the optimal concentration, the value of sample OD minus control OD shall be close to the standard OD. If the enzyme activity of the sample itself is relatively low, the undiluted stock solution can be tested directly. In addition, the sample dosage for enzymatic reaction can be increased or the enzymatic reaction time can be prolonged appropriately.

Calculation

1. Calculation for Liquid Samples

Definition: One enzyme activity unit is defined as the hydrolysis of 1 nmol maltase per milliliter of sample per minute at 37 °C and pH 6.0.

Calculation formula:

$$\text{Lactase activity(U/mL)} = \frac{A_{Assay} - A_{Control}}{A_{Standard} - A_{Blank}} \times C_{Standard} \div 2 \times V_{Total} \div T \div \frac{10^6}{V_{Sample}}$$

2. Calculation for Tissue (or Cell) Samples

Definition: One enzyme activity unit is defined as the hydrolysis of 1 nmol maltase per milligram of tissue protein per minute at 37 ℃ and pH 6.0.

Calculation formula:

$$\text{Lactase activity(U/mg)} = \frac{A_{Assay} - A_{Control}}{A_{Standard} - A_{Blank}} \times C_{Standard} \div 2 \times V_{Total} \div T \div \frac{10^6}{Cpr \times V_{Sample}}$$

Annotation:

$C_{Standard}$: Standard solution concentration, 1.85mmol/L

V_{Total}: Total volume of enzymatic reaction, 0.1×10^{-3}L

V_{Sample}: Sample dosage, 0.025 mL

T: Reaction time, 20 min

Cpr: Sample protein concentration, mg/mL

10^6: Conversion of mmol to nmol

2: Indicates that one maltose molecule is decomposed into two glucose molecules.

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. This kit can be used 100 times when measured with a microplate reader, and 25 times when using a spectrophotometer.