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

Trehalase (THL) Assay Kit

Catalog No.: BC00200

Size: 50T

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	Trehalase (THL) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell, bacterial
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (505 nm)

Product Introduction

Trehalase (THL) is widely distributed in animals, plants, microorganisms and cultured cells. The main physiological function of trehalase is to decompose trehalose into glucose in organisms, which is directly utilized for energy supply.

Principle

THL catalyzes the hydrolysis of trehalose to produce glucose. Glucose is then oxidized by glucose oxidase to form gluconic acid and hydrogen peroxide. Under the catalysis of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine coupled with phenol to generate a chromogenic product. The absorbance is measured at 505 nm to reflect the activity of THL.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	10 mL	Store at 4°C
Reagent 2	Liquid	25 mL	Store at 4°C
Reagent 3	Liquid	25 mL	If freezing occurs, thaw the solution in a 37°C water bath before use. Store at 4°C.
Reagent 4	Extraction solution	30 mL	Store at 4°C

Storage

The reagents can be stored at 4°C for up to 12 months with stability.

Preparation

Visible spectrophotometer, water bath pot, adjustable pipette, 1 mL glass cuvette, mortar, ice and distilled water.

- **Sample handling**

1. Tissue sample: Homogenize the tissue in an ice bath at a ratio of tissue fresh weight (g) to Extraction solution (mL) = 1:5-10 (it is recommended to weigh 0.1 g of tissue and add 1 mL of Extraction solution). Centrifuge at 8000 g and 4°C for 10 min, then collect the supernatant and place it on ice for subsequent determination.
2. Serum (plasma) sample: According to the ratio of serum (plasma) to Extraction solution of 1:5-10 (it is recommended to add 0.1 mL of serum into 1 mL of Extraction solution), homogenize in an ice bath, centrifuge at 8000 g and 4°C for 10 min, then collect the supernatant and place it on ice for subsequent measurement.
3. Bacterial or Cell Samples: Collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant. Prepare at a ratio of 500-1000 ten thousand bacteria/cells per mL extraction solution (recommended: add 5 million bacteria/cells into 1 mL of extraction solution). Perform ultrasonic disruption in an ice bath at a power of 20% or 200 W: sonicate for 3 s with a 10 s interval, repeat 30 times. Afterwards, centrifuge at 8000 g and 4°C for 10 min, then collect the supernatant and place it on ice for subsequent testing.

Operation process

1. Working solution preparation: Mix Reagent 2 and Reagent 3 in equal proportions. Prepare an appropriate amount as needed and use it immediately after preparation.

	Assay tube	Control tube
Supernatant (μL)	90	90
Reagent 1 (μL)	150	

Distilled water (μL)		150
Incubate accurately in a water bath at 45 °C for 15 min, then terminate the reaction by heating in a 95 °C water bath for 5 min. Centrifuge at 10000 g for 10 min, take 100 μL of the supernatant, and then add the working solution.		
Supernatant (μL)	100	100
Working solution (μL)	900	900
Mix well, incubate in a constant temperature water bath at 37 °C for 15 min, then measure the absorbance value A at a wavelength of 505 nm. Then calculate $\triangle A = A_{Assay} - A_{Control}$.		

Calculation

1. Calculated according to protein concentration

   Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol glucose per minute per mg of tissue protein.

$$\text{THL Activity (U/mg)} = \frac{(\Delta A + 0.0042) \times V_{Total} \times 1000}{0.4858 \times V_{Sample} \times Cpr \times T}$$

2. Calculated based on sample fresh weight

   Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of glucose per minute per gram of tissue sample.

$$\text{THL Activity (U/g)} = \frac{(\Delta A + 0.0042) \times V_{Total} \times V_{Extract} \times 1000}{0.4858 \times V_{Sample} \times W \times T}$$

3. Calculated by the density of bacterial or cell samples

   Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol glucose per minute per ten thousand bacteria or cells.

$$\text{THL Activity } (U/10^4) = \frac{(\Delta A + 0.0042) \times V_{Total} \times V_{Extract} \times 1000}{0.4858 \times V_{Sample} \times 500 \times T}$$

4. Calculated based on serum (plasma) volume

   Definition: One unit of enzyme activity is defined as the production of 1 nmol of glucose catalyzed per minute per milliliter of serum (plasma).

$$\text{THL Activity } (U/mL) = \frac{(\Delta A + 0.0042) \times V_{Total} \times V_{Extract} \times 1000}{0.4858 \times V_{Sample} \times V \times T}$$

Annotation:

V: Serum (plasma) volume, 0.1 mL

$V_{Extract}$: Total volume of extraction solution, 1 mL

V_{Total}: Total volume of enzymatic reaction, 0.24 mL

V_{Sample}: Sample volume, 0.09 mL

W: Sample mass, g

T: Reaction time, 10 min

Cpr: Sample protein concentration, mg/mL, shall be determined separately

500: Total number of cells or bacteria, unit in ten thousand; the quantity is 5 million

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.