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

Trehalose Content Assay Kit

Catalog No.: BC00199

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	Trehalose Content Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell, bacterial
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (620 nm)

Product Introduction

Trehalose is widely present in animals, plants, microorganisms and cultured cells. It possesses unique biological characteristics not found in other carbohydrates, and can protect organisms from damage under harsh stress conditions such as drought, high temperature, dehydration, freezing, high osmotic pressure and toxic environments.

Principle

Taking advantage of the extraction specificity of trichloroacetic acid for trehalose, trehalose can be isolated from samples, followed by quantitative detection using the anthrone method.

Components

No.	Components	Size	Notes
Reagent 1	Extraction solution	50 mL	Store at 4°C
Reagent 2	Chromogenic powder	1 vial	Store at 4°C, add 7.5 mL of water before use, then slowly add 42.5 mL of concentrated sulfuric acid while stirring continuously until fully dissolved. Unused reagent can be stored at 4 °C for one week.
Reagent 3	Trehalose Standard Powder	10 mg	Store at 4°C

Notes:

Preparation of 10 mg/mL standard solution: Dissolve one vial of standard powder with 1 mL of distilled water to prepare a 10 mg/mL standard solution, which can be stored at 4°C for 1 month.

Preparation of 0.04 mg/mL standard solution: Dilute the 10 mg/mL standard solution with distilled water at a ratio of 1:249 (250-fold dilution) to obtain a 0.04 mg/mL standard solution. For example, take 40 μL of 10 mg/mL standard solution and add distilled water to make a final volume of 10 mL. Prepare freshly before use.

Storage

The reagents can be stored at 4°C for up to 6 months with stability.

Preparation

Visible spectrophotometer, water bath, adjustable pipette, 1 mL glass cuvette, mortar, concentrated sulfuric acid and distilled water.

- **Sample handling**

1. Tissue sample: Homogenize the tissue in an ice bath at a ratio of tissue weight (g) to extract volume (mL) of 1:5-10 (it is recommended to weigh 0.1 g of tissue and add 1 mL of extract). Allow to stand at room temperature for 45 minutes with shaking 3-5 times during incubation. Centrifuge at 8000 g at room temperature for 10 min, and collect the supernatant for measurement.
2. Serum (plasma) sample: Mix serum (plasma) with extract at a ratio of 1:5-10 (it is recommended to add 1 mL of extract to 0.1 mL of serum). Vortex thoroughly, stand at room temperature for 45 minutes, and shake 3-5 times during this period. Centrifuge at 8000 g at room temperature for 10 min, then collect the supernatant for subsequent detection.
3. Bacterial or Cell Samples: Collect bacteria or cells into a centrifuge tube and discard the supernatant after centrifugation. Mix the sample at a ratio of 500-1000 (ten thousand cells/bacteria): 1 mL extract (it is recommended to add 1 mL of extract to 500 ten thousand bacteria or cells). Perform ultrasonic disruption in an ice bath: power 20% or 200 W, ultrasonic duration 3 s, interval 10 s, repeat 30 times. Allow the mixture to stand at room temperature for 45 minutes and shake 3-5 times during the period. Centrifuge at 8000 g at room temperature for

10 min, then collect the supernatant for subsequent determination.

Operation process

	Blank tube	Standard tube	Assay tube
Reagent 1 (μL)	200		
0.04 mg/mL standard solution (μL)		200	
Supernatant (μL)			200
Reagent 2 (μL)	800	800	800
Mix well, seal tightly, and incubate in a 95 °C water bath for 10 min. Cool with running water, then vortex thoroughly. Use a 1 mL cuvette with 1 cm optical path, and measure the absorbance (A) of each tube at 620 nm with a spectrophotometer.			

Notes:

1. Prior to the formal experiment, select two samples with expected large differences to perform a concentration gradient test to explore the optimal sampling concentration.
2. If the absorbance value is greater than 1, dilute the sample for determination and multiply the result by the dilution factor during calculation.

Calculation

1. Calculation for serum (plasma) samples

$$\text{Trehalose Content (mg/mL)} = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \times V_{Extract} \div V \times N$$

2. Calculation for tissue samples

$$\text{Trehalose Content (mg/g)} = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \times V_{Extract} \div W \times N$$

3. Calculation for tissue or bacterial (cell) samples

$$\text{Trehalose Content (mg/10}^4\text{)} = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \times V_{Extract} \div 10^4 \times N$$

Annotation:

$C_{Standard}$: Standard solution concentration, 0.04 mg/mL

$V_{Extract}$: Volume of extract, 1 mL

V: Serum (plasma) volume, 0.1 mL

W: Sample mass, g

N: Dilution factor of supernatant before detection

10^4: Total cell count

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. When preparing Reagent 2, concentrated sulfuric acid must be added slowly with continuous stirring. Do not rush the operation; otherwise, the reagent may fail to dissolve completely or the liquid may splash and cause personal injury.
4. Wear gloves throughout the operation and avoid exposure to light.
5. After the reaction is completed, mix each tube thoroughly before colorimetric measurement.
6. For plant samples, grind the material into powder with liquid nitrogen first, then weigh the powder, add the extract, mix well for extraction, and proceed with the subsequent steps. However, plant samples with high water content shall be handled according to the operating procedure for tissue samples.
7. If a sulfuric acid-resistant microplate is available, the reaction solution can be transferred into the microplate, and the absorbance can be read at 620 nm using a microplate reader.