Ver.251201

Trehalose Content Assay Kit

BC12005-01(50T/48S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Trehalose is found in a large number of organisms, including bacteria, algae, yeast, plants, insects, and other invertebrates. As trehalose has unique biological characteristics different from other carbohydrates, it can protect organisms cell proteins, fats, sugars, nucleic acids and other groups in harsh environments such as drought, high temperature, dehydration, freezing, high osmotic pressure and toxic substances points are not impaired.

The measurement method is anthrone colorimetric method. It has the advantages of high sensitivity, simple and fast, and suitable for the determination of trace samples. However, the anthrone colorimetric method also has certain defects. If the sample contains soluble sugar, it will affect the determination.
This kit is recommended for determination of samples that do not contain soluble sugar other than trehalose.

Kit components

Reagent	Volume	Storage
Extract solution	60mL	2-8°C
Reagent I	Powder x 2	2-8°C
Reagent II	15mL	2-8°C
Standard	Powder x 1	2-8°C

Solution Preparation:

1. Preparation of working solution: Before use, take 1 bottle of reagent I and add 6.5 mL of reagent II (about 30 T), and use after fully dissolved. Unused reagents can be stored at 2-8°C for a week.

2. Standard: Before use, add 1 mL distilled woer to fully dissolve and prepare 10 mg/mL trehalose standard solution for use; store the inexhaustible reagent at 2-8°C for 4 weeks.

3. Preparation of 0.05 mg/mL glucose standard: 50 μL of 10 mg/mL. glucose standard was added into 950 μL of distilled water, fully mixed and prepared. Then 100μL of 0.5mg/mL glucose standard was added to 900μL of distilled water, that is 0.05mg/ml. glucose standard. (Each tube needs 250μl in the test. In order to reduce the test error, a large volume is prepared).

Reagents and Equipment Required but Not Provided

Spectrophotometer, water bath, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, 1mL glass cuvette, Concentrated sulfuric acid (>95%,AR) and distilled water.

Operation Procedures

I. Sample Preparation

(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. **Tissue sample:** Add extract solution according to the ratio of tissue mass (g): extract solution volume (mL) = 1:5~10 (it is recommended to weigh 0.1g sample and add 1.0 ml. extract solution), after ice bath homogenization, leave it at room temperature for 45 minutes, shake 3-5 times. After cooling, centrifuge at room temperature, 8000g for 10min, take supernatant for test.

2. Bacteria or cell: Collect bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation. The ratio of bacteria/cell amount (104): the volume of extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s, interval 10 s, repeat 30 times). leave it at room temperature for 45 minutes, shake 3-5 times. After cooling, centrifuge at room temperature, 8000g for 10min, take supernatant for test.

3. serum (plasma): Absorb about 100 μL of serum (plasma) and add 0.9 mL of extract solution, leave it at room temperature for 45 minutes, shake it 3~5 times. After cooling, centrifuge at room temperature, 8000g for 10min, take supernatant for test.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 620 nm and set zero with distilled water.
2. Adjust the water bath to 2-8°C
3. Operation table

Reagent (μL)	Test tube (A_T)	Standard tube (As)	Blank tube (A_B)
Sample	250	-	-
Standard	-	250	-
Distilled water	-	-	250
Working solution	210	210	210
Concentrated sulfuric acid	790	790	790
Fully mixed and placed in a 95°C water bath for 10 minutes (Wrap the sealing film to prevent bursting). Add 1 mL mixture into 1mL glass cuvette, and detect the absorbance value of each tube at 620 nm. ΔA_T - A_T-A_B, ΔA_S = A_S-A_B, ΔA_S = A_S-A_B. (The blank and standard tubes only need to be measured 1-2 times)			

Note:

1. It is recommended to use spiral port or locking EP tube to prevent water loss.
2. The working solution and concentrated sulfuric acid can not be mixed in advance.

Calculation

1. Calculate by sample mass

Trehalose Content (mg/g mass) =

$$C_S \times \Delta A_T \div \Delta A_S \times V_T \div (W \times V_T \div V_E) \times F = 0.05 \times \Delta A_T \div \Delta A_S \div W \times F$$

2. Calculate by sample protein concentration

Trehalose Content (mg/mg prot) =

$$C_S \times \Delta A_T \div \Delta A_S \times V_T \div (V_T \div Cpr) \times F = 0.05 \times \Delta A_T \div \Delta A_S \div Cpr \times F$$

3. Calculate by the number of bacteria or cells

Trehalose Content (μg /10^4 cell) =

$$(1000 \times C_S \times \Delta A_T \div \Delta A_S \times V_T) \div (N \times V_T \div V_E) \times F = 0.05 \times \Delta A_T \div \Delta A_S \div N \times F$$

4. Calculate by liquid volume

Trehalose Content (mg/mL) = $C_S \times \Delta A_T \div \Delta A_S \times V_T \div (V_L \times V_T \div V_E) \times F = 0.05 \times \Delta A_T \div \Delta A_S \times F$

1000: Unit conversion coefficient, 1mg / mL = 1000μg/mL
V_T : Sample volume, 0.25mL
V_E : Volume used in the extraction solution, 1mL
V_L: Serum (slurry) volume, 0.1mL
Cpr: Sample protein concentration, mg/mL
W: Fresh weight of sample, g
N: The number of cells or bacteria, count by 10^4

NOTE

1. Concentrated sulfuric acid is highly corrosive, please operate with caution

2. The reagent II is highly volatile, and can be wound after use to seal the sealing film to prevent the reagent from volatilizing too fast.

3. If you need to calculate by sample protein concentration, you need to re-extract the protein from another sample for determination.

4. If $\Delta A_T < 0.03$ it is recommended to increase the sample size before re-determination; if $\Delta A_T > 1$ it is recommended to dilute the sample with distilled water for determination, and synchronous modification calculation formula.