

Trehalose Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0335

Size:100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 100 mL×1	2-8°C
Powder I	Powder×2	2-8°C
Standard	Powder×1	2-8°C

Solution Preparation:

1. Standard: 10 mg of trehalose. Storage at 2-8°C. Add 1 mL of distilled water to the standard before use. The solution concentration is 10 mg/mL, and the reagent can be stored for two weeks at 2-8°C.

2. Preparation of working solution: Before use, take 1 bottle of Reagent I and add 3.5 mL of distilled water, slowly add 14 mL of concentrated sulfuric acid, stir continuously, dissolve fully, and set aside. Unused reagents can be stored at 2-8°C for a week, and cannot be used after the color of the reagent becomes darker.

Product Description:

Trehalose is found in a large number of organisms, including bacteria, algae, yeast, plants, insects, and other invertebrates. Because 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.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/Microplate reader, water bath, adjustable transferpeltor, sonicator, mortar/homogenizer, centrifuge, micro glass cuvette/96-well plate, concentrated sulfuric (H₂SO₄) acid(>95%, AR) and distilled water.

Sample preparation:

1. Bacterial or cell processing: Collect bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation. Add 1 mL of extract solution into 5 million bacteria or cells, ultrasonically break the bacteria or cells (power 200W, ultrasonic 3 seconds, interval 10 seconds, repeat 30 times), stand at

room temperature for 45 minutes, shake 3 to 5 times. After cooling, centrifuge at 8000 ×g at room temperature for 10 minutes, take the supernatant.

2. Tissue processing: Weigh about 0.1 g of sample, grind it at room temperature, add 1 mL of Extract solution, leave it at room temperature for 45 minutes, shake 3 to 5 times. After cooling, centrifuge at 8000×g at room temperature for 10 minutes, and take the supernatant.
3. Serum (plasma): Absorb about 100 μL of serum (plasma), add 0.9 mL of Extract solution, leave it at room temperature for 45 minutes, shake it 3 to 5 times. After cooling, centrifuge at 8000 ×g at room temperature for 10 minutes, and take the supernatant.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 620 nm and spectrophotometer set the counter to zero with distilled water. adjust the water bath to 95°C.
2. Standard solution: diluted to 0.2、 0.1、 0.075、 0.05、 0.025、 0.0125、 0 mg/mL with distill water.
3. Establishment of standard curve: Take 0.06 mL of standard solution and 0.24 mL of working solution into a EP tube, 95°C water bath for 10 minutes (close tightly to prevent water loss), naturally cool to room temperature, Take 200 μL to micro glass cuvette/96-well flat-bottom plate to measure the absorbance at 620 nm. $\Delta A_s = A_s - A_B$, according to the concentration of the standard tube (x, mg/mL) and absorbance ΔA_s (y, ΔA_s), establish a standard curve.
4. Sample measurement: take 0.06 mL of sample and 0.24 mL of working solution into EP tube, 95°C water bath for 10 minutes (close tightly to prevent water loss), naturally cool to room temperature. Take 200 μL to micro glass cuvette/96-well flat-bottom plate, measure absorbance A at 620 nm, Denoted as A_T , calculate $\Delta A_T = A_T - A_B$.

III. Calculation:

1. According to the standard curve, bring the ΔA_T (y, A_T) into the formula to calculate the sample concentration (x, mg/mL).

2. Protein concentration:

$$\text{Trehalose (mg/g prot)} = V1 \times x \div (Cpr \times V1) = x \div Cpr$$

3. Sample weight:

$$\text{Trehalose (mg/g sample)} = V1 \times x \div (W \times V1 \div V2) = x \div W$$

4. Cells or bacteria:

$$\text{Trehalose } (\mu\text{g}/10^4 \text{ cell}) = 1000 \times V1 \times x \div (500 \times V1 \div V2) = 2 \times x$$

5. Liquid volume:

$$\text{Trehalose (mg/mL)} = V1 \times x \div (V3 \times V1 \div V2) = 10 \times x$$

1000: 1 mg/mL = 1000 μg/mL;

V1: Sample volume, 0.06 mL;

V2: Volume used in the extraction solution, 1 mL;

V3: Serum (slurry) volume, 0.1 mL

Cpr: Sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

500: The number of cells or bacteria, 5 million.

Note:

If the absorbance is greater than linear range absorbance, dilute the sample with the Extract solution before measuring, and multiply it by the corresponding dilution factor in the calculation formula.

Related publications:

[1] Qin L, Wang L, Guo Y, et al. An ERF transcription factor from *Tamarix hispida*, ThCRF1, can adjust osmotic potential and reactive oxygen species scavenging capability to improve salt tolerance[J]. *Plant Science*, 2017, 265: 154-166.

References:

[1] Al-Naama M, Ewaze J O, Green B J, et al. Trehalose accumulation in *Baudouinia compniacensis* following abiotic stress[J]. *International Biodeterioration & Biodegradation*, 2009, 63(6): 765-768.

Related products:

BC0230/BC0235 Reducing sugar detection kit

BC2500/BC2505 Glucose detection kit

BC0030/BC0035 Plant soluble sugar content detection kit

BC2710/BC2715 Total sugar content detection kit

BC4280/BC4285 Cellulose (CLL) content detection kit

BC4390/BC4395 D- xylitose content detection kit

Technical Specifications:

The detection limit:0.0055 mg/mL

The Linear range: 0.00625-0.4 mg/mL