Trehalose Content Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: NA0599

Size:100T/96S

Product composition:

Extract reagent: Liquid 100 mL×1. Storage at 4℃.

Reagent I: Powder×1. Storage at 4℃.

Standard: Powder×1, 10mg of trehalose. Storage at 4℃. The standard product is dissolved in 1mL of distilled water, and the solution concentration is 10mg / mL.

Preparation of working solution: Adding 7 mL of distilled water to each bottle of reagent I and then add 28mL of concentrated sulfuric acid slowly, keep stirring, fully dissolve. Unused reagent can stored at 4℃ for one week.

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 transferpettor, mortar/homogenizer, centrifuge, micro glass cuvette/96-well plate, concentrated sulfuric (H_2SO_4) acid and distilled water.

Procedure:

I. 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 20%/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, 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, 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, and take the supernatant.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 620 nm and set the counter to zero with distilled water. Adjust the water bath to 95℃.
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℃ 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. Establish a standard curve based on the concentration (y) and absorbance (x) of the standard sample.
4. Sample measurement: take 0.60 mL of sample and 0.24 mL of working solution into EP tube, 95℃ 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.

III. Calculation:

1. Calculate the trehalose content y (mg / mL) in the sample according to the standard curv**e.**
2. Protein concentration:

Trehalose (mg/g prot)= V1×y÷(Cpr×V1) = y÷Cpr

3. Sample weight:

Trehalose (mg/g sample) = V1×y÷(W×V1÷V2) = y÷W

4. Cells or bacteria:

Trehalose (μg/10^4 cell) = 1000×V1× y÷(500×V1÷V2) =2×y

5. Liquid volune:

Trehalose (mg/mL) = V1×y÷(V3×V1÷V2) = 10y

1000: 1 mg/mL = 1000μg/mL;
V1: Sample volume, 0.06mL;
V2: Volume used in the extraction solution, 1mL;
V3: Serum (slurry) volume, 0.1mL
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 Baudoinia compniacensis following abiotic stress[J]. International Biodeterioration & Biodegradation, 2009, 63(6): 765-768.

Related products:

NA0851/NA0609 Reducing sugar detection kit
NA0692/NA0451 Glucose detection kit
NA0869/NA0626 Plant soluble sugar content detection kit
NA0673/NA0432 Total sugar content detection kit
NA0320/NA0319 Cellulose (CLL) content detection kit
NA0294/NA0293 D- xylitose content detection kit

Technical Specifications:

The detection limit:0.0055 mg/mL
The Linear range: 0.00625-0.4 mg/mL