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Trehalose Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0295

Size:50T/48S

Product composition:

Extract reagent: Liquid 50 mL×1. Storage at 4℃ .

Reagent I: Powder×1. Storage at 4℃ .

Standard: Powder×1, 10 mg 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 16 mL of distilled water to each bottle of Reagent I and then add 64 mL 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. 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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, adjustable transferpettor, mortar/homogenizer, centrifuge, ice, 1 mL glass cuvette, 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%, 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 and 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 and room temperature, and take the supernatant.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 620 nm and set the counter to zero with distilled water.
2. Adjust the water bath to 95℃ .
3. Standard solution: Diluted to 0. 1, 0.08, 0.06, 0.04, 0.02, and 0 mg / mL with distilled water.
4. Establishment of standard curve: Take 0.25 mL of standard solution and 1 mL of working solution into an EP tube, 95℃ water bath for 10 minutes (close tightly to prevent water loss), naturally cool to room temperature, take 1 mL to measure the absorbance at 620 nm. Establish a standard curve based on the concentration (y) and absorbance (x) of the standard sample.
5. Sample measurement: take 0.25 mL of sample and 1 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 1 mL to cuvette, measure absorbance A at 620 nm.

III. Calculation:

1. Calculate the trehalose content y (mg / mL) in the sample according to the standard curve.
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 = 1000 μg/mL;
V1: Sample volume, 0.25 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 Baudoinia compniacensis following abiotic stress[J]. International Biodeterioration & Biodegradation, 2009, 63(6): 765-768.

Related products:

AK0315/AK0314 Reducing sugar detection kit

AK0221/AK0219 Glucose detection kit

AK0663/AK0613 Plant soluble sugar content detection kit

AK0183/AK0182 Total sugar content detection kit

AK0090/AK0089 Cellulose (CLL) content detection kit

AK0072/AK0070 D- xylitose content detection kit

Technical Specifications:

The detection limit:0.0016 mg/mL

The Linear range: 0.003125-0. 1 mg/mL