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CheKine™ Micro Trehalose Content Assay Kit

Cat #: KTB1335 Size: 48 T/96 T

	Micro Trehalose Content Assay Kit		
REF	**Cat #:** KTB1335	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissue, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Trehalose is widely found in animals, plants, microorganisms and cultured cells. Because trehalose has unique biological characteristics different from other carbohydrates, it can protect the protein, fat, sugar, nucleic acid and other components of biological cells from damage under the harsh environment such as drought, high temperature, dehydration, freezing, high osmotic pressure and toxic substances. CheKine™ Micro Trehalose Content Assay Kit can be used to detect biological samples such as animal and plant tissue, cells or bacteria, plasma, serum or other liquid samples. In the kit, the method was determined by anthrone colorimetric method. It has the advantages of high sensitivity, simple and fast, and is suitable for the determination of trace samples. However, anthrone colorimetric method also has some defects, if the sample contains soluble sugar, it will affect the determination. **This kit is recommended for the determination of samples that do not contain soluble sugars other than trehalose.**

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 620 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, concentrated sulfuric acid
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ: Prepared before use. After add 1.875 mL deionized water for 48 T and 3.75 mL eionized water for 96 T, **slowly** add 10.625 mL concentrated sulfuric acid for 48 T and 21.25 mL concentrated sulfuric acid for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 1 week.

Note: Reagent Ⅰ is highly corrosive, please use caution.

Standard: Prepared before use; Add 1 mL deionized water to fully dissolve, that is 10 mg/mL trehalose standard; Store at 4℃, protected from light for 2 weeks.

1 mg/mL trehalose Standard preparation: Prepare 1 mg/mL trehalose Standard by diluting 100 μL 10 mg/mL trehalose Standard into 900 μL Deionized Water. Use the 1 mg/mL trehalose standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	200 μL of 1 mg/mL Standard	800	0.2
Std.2	500 μL of Std.1 (0.2 mg/mL)	500	0.1
Std.3	150 μL of Std.2 (0.1 mg/mL)	50	0.075
Std.4	100 μL of Std.2 (0.1 mg/mL)	100	0.05
Std.5	50 μL of Std.2 (0.1 mg/mL)	150	0.025
Std.6	25 μL of Std.2 (0.1 mg/mL)	175	0.0125
Blank	0	200	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Stand at room temperature for 45 min, oscillate 3~5 times,Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Stand at room temperature for 45 min, oscillate 3~5 times,Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.
3. Plasma, Serum or other Liquid samples: Take 0.1 mL liquid, add 1 mL Extraction Buffer and homogenize or mortar on ice. Stand at room temperature for 45 min, oscillate 3~5 times,Centrifuge at 8,000 g for 10 min at 25℃. Use supernatant for assay.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 620 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	60
Standard	0	60	0
Deionized water	60	0	0
Reagent Ⅰ	240	240	240

3. Mix well, water bath at 95℃ for 10 min (cover tightly to prevent water loss), natural cooling to room temperature, take 200μL into a 96-well plate or microglass cuvette. Detect the absorbance at 620 nm. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test} is greater than 1.0 or ΔA_{Test} exceeds the absorption value of the linear range, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x (mg/mL).

2. Calculation of the trehalose content

(1) Calculated by protein concentration

Trehalose (mg/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)$**=x÷Cpr**

(2) Calculated by fresh weight of samples

Trehalose (mg/g fresh weight)=$(V_{Sample}\times x)\div(W\times V_{Sample}\div V_{Total\ sample})$**=x÷W**

(3) Calculated by bacteria or cells

Trehalose (μg/10^4)=$(1{,}000\times V_{Sample}\times x)\div(n\times V_{Sample}\div V_{Total\ sample})$**=1,000x÷n**

(4) Calculated by volume of liquid samples

Trehalose (mg/mL)=$(V_{Sample}\times x)\div(V_{Liquid}\times V_{Sample}\div V_{Total\ sample})$**=10x**

V_{Sample}: Added the sample volume, 60 μL=0.06 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL; V_{Liquid}: Added the plasma, serum or other liquid samples volume, 0.1 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; 1,000: 1 mg/mL=1,000 μg/mL; n: Number of bacteria or cells, calculated in units of ten thousand.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

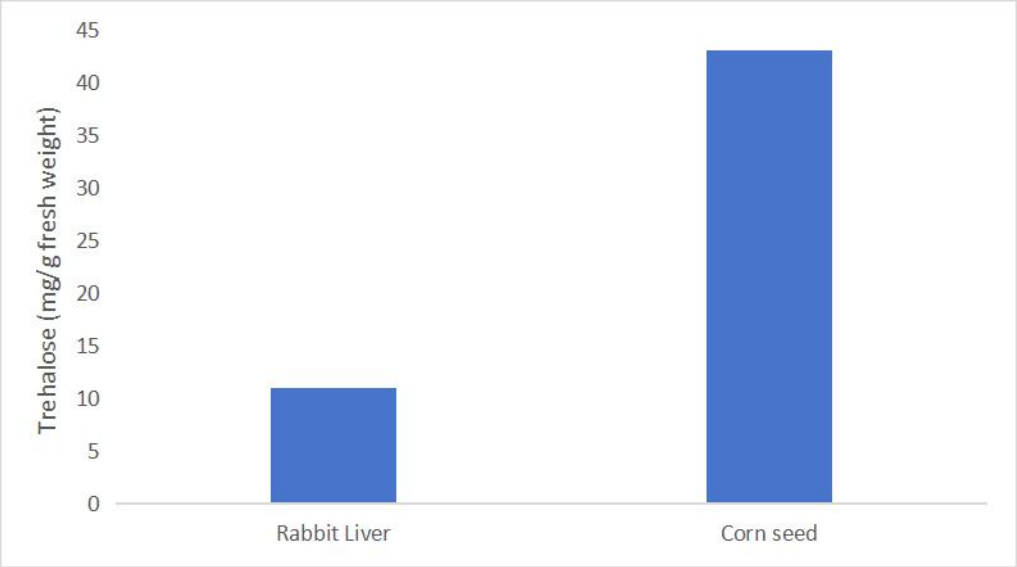

Figure 1. Determination of trehalose content in rabbit liver and corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.