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CheKine™ Micro Sorbitol Content Assay Kit

Cat #: KTB3061 Size: 48 T/48 S 96 T/96 S

	Micro Sorbitol Content Assay Kit		
REF	**Cat #:** KTB3061	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Sorbitol is widely found in animals, plants, microorganisms and cultured cells. It's a form of sugar transport, and also closely related to biological resistance and food flavor. Therefore, it is often necessary to detect the changes of sorbitol content in sugar metabolism, stress resistance and food research. CheKine™ Micro Sorbitol Content Assay Kit can be used to detect biological samples such as animal and plant tissues, serum or plasma. In the kit, sorbitol can form blue complex with copper ions in alkaline solution, which has characteristic absorption peaks at 655 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	2.5 mL	5 mL	4℃
Reagent Ⅱ	2.5 mL	5 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 655 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL eppendorf tube
- Water bath pot, centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Standard: Prepared before use. Add 1 mL of deionized water to dissolve it into 10 mg/mL standard solution, which could be stored at 4℃, protected from light for 2 weeks.

Standard preparation: Using 10 mg/mL Sorbitol Standard, prepare standard curve dilution as described in the table:

Num.	Standard volume	Deionized water volume (μL)	Concentration (mg/mL)
Std.1	400 μL 10 mg/mL Standard	600	4
Std.2	500 μL of Std.1 (4 mg/mL)	500	2
Std.3	500 μL of Std.2 (2 mg/mL)	500	1
Std.4	500 μL of Std.3 (1 mg/mL)	500	0.5
Std.5	500 μL of Std.4 (0.5 mg/mL)	500	0.25
Std.6	500 μL of Std.5 (0.25 mg/mL)	500	0.125
Blank	0	500	0

Note: Always prepare fresh standards per use; Diluted Standard 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 deionized water and homogenize. Water bath at 95℃ for 10 min. (cover tightly to prevent moisture loss.) After cooling, 8000 g, centrifuge 10 min at room temperature, the supernatant was taken to be tested.
2. Plasma, Serum or other Liquid samples: Direct detection.

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 655 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 1.5 mL eppendorf tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Sample supernatant	0	0	230
Standards	0	230	0
Reagent Ⅰ	35	35	35
Reagent Ⅱ	35	35	35
Deionized Water	230	0	0

3. Mix well, rest at room temperature for 15 min, then 8000 g, centrifuge 10 min at room temperature. Take 200 μL supernatant to a microglass cuvette or 96-well plate, the absorbance value is measured at 655 nm. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, and 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: Standard curves and blank holes only need to be tested 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 4 mg/mL of $\Delta A_{Standard}$, the sample can be appropriately diluted with

deionized water, 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. The determination of ΔA_{Test} is brought into the equation to get x(mg/mL).

2. Calculation of the sorbitol content

(1) Calculated by sample protein concentration

Sorbitol(mg/mg prot)=$y \times V_{Sanple} \div (V_{Sanple} \times Cpr)$**=y÷Cpr**

(2) Calculated by fresh weight of samples

Sorbitol(mg/g fresh weight)=$y \times V_{Sanple} \div (W \times V_{Sanple} \div V_{Water})$**=y÷W**

(3) Calculated by volume of liquid samples

Sorbitol(mg/L)**=x×n**

V_{Sanple}: added sample volume, 0.23 mL; V_{Water}: added deionized water volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; n: the sample dilution factor of serum and other liquid samples.

Precautions

The precipitation can not be produced in the operation process of the experimental step, and the sample can be mixed at the same time or the order of sample can be changed.

Typical Data

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

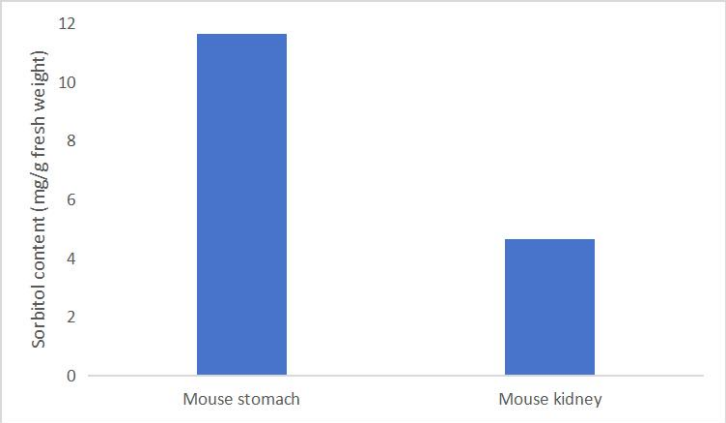

Figure 1. Determination of sorbitol groups in mouse stomach and kidney by this kit

Recommended Products

Catalog No.	Product Name
KTB1330	CheKine™ Micro Blood sugar Content Assay Kit
KTB1340	CheKine™ Micro Glycogen Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.