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CheKine™ Micro Total Polysaccharide Content Assay Kit

Cat #: KTB1351 Size: 48 T/96 T

	Micro Total Polysaccharide Content Activity Assay Kit		
REF	**Cat #:** KTB1351	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Polysaccharide are ubiquitous substances in organisms, which are a kind of natural polymers connected by Aldose or ketose through glycosidic bonds. it is an important biological macromolecule in the organism and one of the basic substances to maintain the normal operation of life activities. CheKine™ Micro Total Polysaccharide Content Assay Kit can be used to detect biological samples such as animal and plant tissues. In the kit, the total polysaccharide were extracted by water extraction and alcohol precipitation, and the content of total polysaccharide was determined by phenol-sulfuric acid method.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	6 mL	12 mL	4℃
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 490 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, centrifuge
- Deionized water, absolute ethanol, concentrated sulfuric acid
- Homogenizer or mortar

Reagent Preparation

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

Reagent Ⅱ: Absolute ethanol. **(Required but not provided)**

Reagent Ⅲ: Concentrated sulfuric acid. **(Required but not provided)**

Standard: Prepared before use. Add 1 mL deionized water to fully dissolve to generate a 10 mg/mL glucose standard solution, store at 4℃ and use within 2 weeks.

Standard preparation: Use the 10 mg/mL glucose 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	15 μL 10 mg/mL Standard	585	0.25
Std.2	300 μL of Std.1 (0.25 mg/mL)	300	0.125
Std.3	300 μL of Std.2 (0.125 mg/mL)	300	0.0625
Std.4	300 μL of Std.3 (0.0625 mg/mL)	300	0.03125
Std.5	300 μL of Std.4 (0.03125 mg/mL)	300	0.015625
Std.6	300 μL of Std.5 (0.0.015625 mg/mL)	300	0.0078125

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.

Tissues: Sample drying and crushing, weighing about 0.05 g sample, adding 1 mL deionized water, fully homogenizing. The extract was extracted in 100℃ water bath for 2 h (the lid must be closed to prevent water loss). After cooling, 10,000 g, 10 min was centrifuged and the supernatant was taken. Absorb the supernatant of 0.2 mL, slowly add 0.8 mL Reagent II, after mixing, sit at 4℃ overnight, 10,000 g centrifugal 10 min, discard the supernatant, add 1 mL deionized water to the precipitation, fully mix and dissolve the precipitation to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 490 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in 1.5 mL EP tube)

Reagent	Standard Well (μL)	Test Well (μL)
Sample	0	200
Standard	200	0
Reagent I	100	100
Reagent III	500	500

3. After mixing, Incubate at 90℃ for 20 min, running water cooling. Add 200 μL in the 96-well plate, detect the absorbance at 490 nm. The Standard Well is marked as $A_{Standard}$, the Test Well is marked as $A_{Test.}$.

Note: 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 less than 0.2, increase the sample quantity appropriately. If A_{Test} is greater than 2, 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 $A_{Standard}$ 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 total polysaccharide content

Calculated by dry weight of samples

Total polysaccharide(μg/g dry weight)=x×V1÷V2×V3÷W×1,000=**5,000x÷w**

V1: Volume after alcohol precipitation and re-dissolution, 1 mL; V2: Volume for alcohol precipitation; 0.2 mL; V3: The volume of water added during extraction, 1 mL; W: Sample weight, g; 1,000: Conversion coefficient, 1 mg=1,000 μg.

Typical Data

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

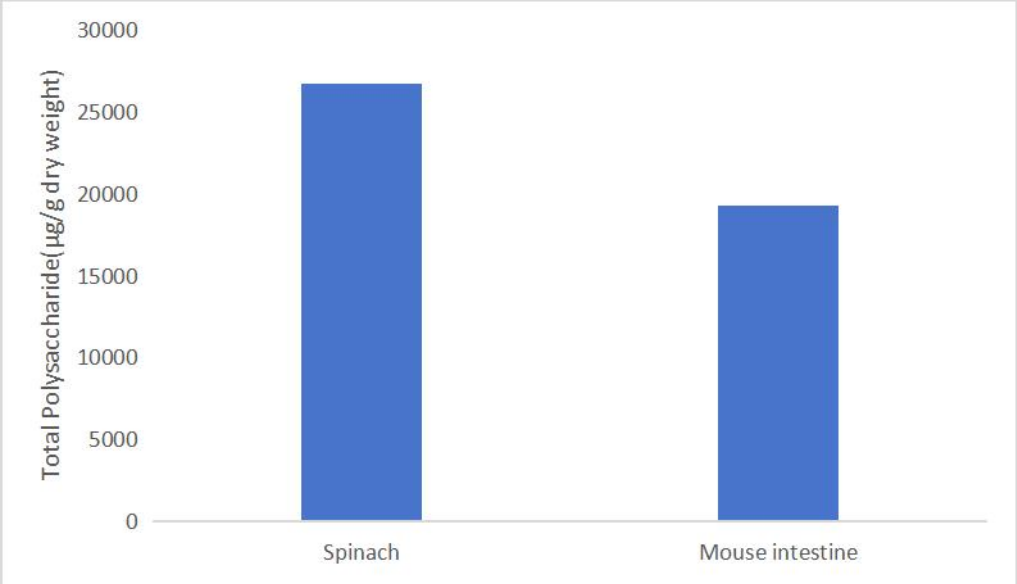

Figure 1. Determination of total polysaccharide content in spinach and mouse intestine by this kit.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1110	CheKine™ Lactate Dehydrogenase (LDH) Activity Assay Kit

Disclaimer

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