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Total Carbohydrate Content Assay kit

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

Operation Equipment: Spectrophotometer / Microplate reader

Cat No: AK0182

Size: 100T/96S

Components:

Reagent I: 100 mL×1 bottle, store at 4℃ .

Reagent II: 100 mL×1 bottle, store at 4℃ .

Reagent III: 5 mL×1 bottle, store at 4℃ .

Standard: Power×1 bottle, 10mg of glucose, store at 4℃ . It is dissolved in 1mLdistilled Water to 10 mg/mL before test.

Description:

Carbohydrate is one of the important constituents of plants and the main raw materials and storage materials in metabolism. Total sugar mainly refers to reducing glucose, fructose, pentose, lactose and sucrose, maltose, and possibly partially hydrolyzed starch that can be hydrolyzed to reducing monosaccharides under measurement conditions.

The total carbohydrate can be acid hydrolyzed into reduced sugar. In the presence of alkaline solution, the DNS reagent is reduced to an amino compound by co-heating with the reduced sugar, which shows orange-red color and has a maximum absorption peak at 540 nm.

Required but not provided:

Spectrophotometer/microplate reader, water bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar, distilled water.

Protocol:

I. The extraction of Soluble sugar

1) Tissue: Add 1mL of Reagent I and 1.5 mL of distilled water to 0. 1g of sample, homogenate. Place in 100℃ water bath for 30min. Add 1 mL of reagent II, mix thoroughly. Then distilled water is made up to 10mL, centrifuge at 8000g for 10min at 25℃ . Take supernatant for test.

2) Liquid Sample: Add 0. 1mL of Reagent I and 0.15 mL of distilled water to 0.1 mL of sample, homogenate. Place in 100℃ water bath for 30min. Add 0.1 mL of Reagent II, mix thoroughly. Then distilled water is made up to 1mL, centrifuge at 8000g for 10min at 25℃ . Take supernatant for test.

Ⅱ. Operation

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 540nm, set zero with distilled water.
2. Standard working solution: 10 mg/mL standard is diluted with distilled water to 1.5， 1, 0.8, 0.6, 0.5, 0.4,

0.2, 0. 1μmol/mL for test.

3. Add reagents according to the following table.

Reagent name (μL)	Blank tube(B)	Test tube(T)	Standard tube (S)
Sample		30	
Distilled water	30	-	
Standard			30
Reagent III	30	30	30
Mix thoroughly, place at 100℃ water bath for 10 min, cool to room temperature.			
Distilled water	180	180	180

Mix thoroughly. Take 200μL to detect the absorbance at 540nm. ΔA=A(T)-A(B), ΔA(S)=A(S)-A(B). Blank tube just only needs to be measured 1-2 times.

Ⅲ. Calculation of Total Carbohydrate

A. Drawing of standard curve.

Standard solution concentration as x axis and its corresponding absorption value (ΔAs) as y axis, the standard equation is y=kx+b. Bring ΔA(T) into the formula to get x (μmol/mL).

B. **Calculation of the content of total carbohydrate:**

1. Sample weight

   Total Carbohydrate (mg /g) = (x ×Vs) ÷W×F=10×x÷W×F.

2. Liquid volume

   Total Carbohydrate(mg/mL) = (x ×V1)÷V2×F=10×x ×F.

Vs: Total sample volume, 10mL

V1: Total liquid sample volume, 1mL.

V2: liquid sample volume, 0. 1mL.

W: Sample weight, g

F: dilution factor.

Note:

1. If ΔA>1.2, please dilute the supernatant with distilled water and multiply the dilution factor in the formula.

2. The degree of cellulose decomposition cannot reach 100% in our kit.

Experimental example:

1. Take 0. 1g of rabbit liver for sample processing, take the supernatant, and operate according to the determination steps. Use 96 well plate to measure and calculate ΔA = A_T-a blank tube = 0.599-0.05 = 0.549, standard curve y = 0.8881x-0.0561, then x = 0.6813.

Total sugar (mg / g mass) = 10×x ÷ W = 68.13 mg/g mass.

2. Take 0. 1g Jasmine for sample processing, take the supernatant, and operate according to the

determination steps. Use 96 well plate to measure and calculate $\Delta A = A_T - A_B = 0.594 - 0.05 = 0.544$, standard curve y = 0.8881x-0.0561, then x = 0.6575

Total sugar (mg/g) = 10×x÷W = 65.75 mg/g.

3. The mouse serum is taken for processing, and the supernatant is taken and operated according to the determination steps. The calculation was made with 96 well plate, $\Delta A = A_T - A_B = 0.294 - 0.05 = 0.244$, and the standard curve y = 0.8881x-0.0561, then x = 0.338.

Total sugar (mg/mL) = 10×x= 3.38 mg/mL.

Related Products:

AK0315/AK0314	Reducing Sugar(RS) Content Assay Kit
AK0223/AK0222	Blood Glucose Content Assay Kit
AK0221/AK0219	Glucose Content Assay Kit
AK0663/AK0613	Plant Soluble Sugar Content Assay Kit

Technical Specifications:

The detection limit: 0.0667 mg/mL

The linear range: 0.09- 1.8 mg/mL