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Total Carbohydrate Content Assay kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0183

Size: 50T/48S

Components:

Reagent I: 50mL×1 bottle, store at 4℃ .

Reagent II: 50mL×1 bottle, store at 4℃ .

Reagent III: 13mL×1 bottle, store at 4℃ .

Standard: Power×1 bottle, 10mg 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, water bath, transferpettor, 1mL glass cuvette, mortar and distilled water.

Protocol:

I. The extraction of Soluble sugar

1) Tissue: Add 1mL of reagent I and 1.5mL 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. 15mL 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 for 30min, adjust wavelength to 540nm, set zero with distilled water.
2. Add reagents according to the following table.
3. Standard working solution: 10mg/mL standard was diluted with distilled water to 1, 0.8, 0.6, 0.5, 0.4, 0.2, 0. 1μmol/mL for test.

Reagent name (μL)	Blank tube(B)	Test tube(T)	Standard tube (S)

Sample		150	
Distilled water	150	-	
Standard			150
Reagent III	150	150	150
Mix thoroughly, place at 100℃ water bath for 10 min, cool to room temperature.			
Distilled water	900	900	900

Mix thoroughly. Detect the absorbance at 540nm. ΔA=A(T)-A(B), ΔA(S)=A(S)-A(B). Blank tube just needs to be conducted 1-2 times.

Calculation of Total Carbohydrate

1. 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).

2. Calculation of the content of total carbohydrate:

A. Sample weight

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

B. Liquid volume

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

Vs: Total sample volume, 10 mL

V1: Total liquid sample volume, 1 mL.

V2: liquid sample volume, 0.1 mL.

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. Measure and calculate $\Delta A = A_T - A_B = 0.965 - 0.019 = 0.946$, standard curve y=1.2984x-0.0284, then x=0.7505.

Total sugar (mg / g mass) = 10×x ÷ W = 75.05 mg/g mass.

2. Take 0. 1g Jasmine for sample processing, take the supernatant, and operate according to the determination steps. Measure and calculate $\Delta A = A_T - A_B = 0.961 - 0.019 = 0.942$, standard curve y=1.2984x-0.0284, then x=0.7474

Total sugar (mg/g) = 10×x÷W = 74.74 mg/g.

3. The mouse serum is taken for processing, and the supernatant is taken and operated according to the

determination steps. Calculation: $\Delta A = A_T - A_B = 0.459 - 0.019 = 0.440$, and the standard curve y=1.2984x-0.0284, then x=0.3608.

Total sugar (mg/mL) = 10×x= 3.608 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: 0.0444 mg/mL

The linear range: 0. 1- 1 mg/mL