Total Carbohydrate Content Assay kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Cat No: BC2710

Size: 50T/48S

Components:

Reagent I: Liquid 50mL×1, store at 2-8℃.

Reagent II: Liquid 50mL×1, store at 2-8℃.

Reagent III: Liquid 13mL×1, store at 2-8℃.

Standard: Power×1, 10mg glucose, store at 2-8℃. It is dissolved in 1mL distilled water to 10 mg/mL before test. The unused reagent can be stored at 2-8℃ for 2 weeks.

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, centrifuge, water bath, transferpettor, 1mL glass cuvette, mortar/homogenizer and distilled water.

Protocol:

1. 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.

2. Operation

1. Preheat spectrophotometer for 30min, adjust wavelength to 540nm, set zero with distilled water.
2. Standard working solution: 10mg/mL standard was diluted with distilled water to 1, 0.8, 0.5, 0.2, 0.1mg/mL for test.
3. Add reagents according to the following table.

Reagent (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. Calculate $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. Blank tube and standard tube just needs to be conducted 1-2 times.

3. Calculation of Total Carbohydrate

1. Drawing of standard curve.

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

2. Calculation of the content of total carbohydrate:

A. Sample weight

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

B. Liquid volume

Total Carbohydrate content (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. The degree of cellulose decomposition cannot reach 100% in this kit.
2. Increase sample volume or dilute sample before determination if the absorbance of test tube exceeds the absorbance in the linear range. And modify the calculation formula.

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 $\Delta A_T = A_T-A_B$ =0.965-0.019=0.946, standard curve y= 1.2984x- 0.0284, then x=0.7505. The result is calculated according to the sample weight:

   Total sugar content (mg/g weight) = 10×x ÷ W = 75.05 mg/g weight.
2. Take 0.1g Jasmine for sample processing, take the supernatant, and operate according to the determination steps. Measure and calculate $\Delta A_T = A_T-A_B$ =0.961-0.019=0.942, standard curve y= 1.2984x-0.0284, then x=0.7474. The result is calculated according to the sample weight:

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

3. The mouse serum is taken for processing, and the supernatant is taken and operated according to the determination steps. Calculation: $\Delta A_T = A_T - A_B$ =0.459-0.019=0.440, and the standard curve y= 1.2984x-0.0284, then x=0.3608. The result is calculated according to the sample volume:

   Total sugar content (mg/mL) = 10×x= 3.608 mg/mL.

Related Products:

BC0230/BC0235	Reducing Sugar Content Assay Kit
BC2490/BC2495	Blood Glucose Content Assay Kit
BC2500/BC2505	Glucose Content Assay Kit
BC0030/BC0035	Plant Soluble Sugar Content Assay Kit

Technical Specifications:

The detection limit: 0.0444 mg/mL

The linear range: 0.1-1 mg/mL