Ver.240707

Total Carbohydrate Content Assay kit

BC12039-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product 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.

Kit components

Reagent	Volume	Storage
Reagent I	50mL× 1	2-8°C
Reagent II	50mL × 1	2-8°C
Reagent III	13mL×1,	2-8°C
Standard	Powder×1, 10mg glucose	2-8°C
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.		

Reagents and Equipment Required but Not Provided

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

Protocol:

1. The extraction of soluble sugar

- **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 30 minutes. Add 1 mL of reagent II, mix thoroughly. Then distilled water is made up to 10 mL, centrifuge at 8000×g for 10 minutes at 25℃. Take supernatant for test.
- **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 30 minutes. Add 0.1 mL of reagent II, mix thoroughly. Then distilled water is made up to 1mL, centrifuge at 8000×g for 10 minutes at 25℃. Take supernatant for test.

2. Operation

1. Preheat spectrophotometer for 30 minutes, 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	Blank tube(B)	Test tube(T)	Standard tube(S)
Sample	-	150 μL	-
Distilled water	150 μL	-	-
Standard	-	-	150 μL
Reagent III	150 μL	150 μL	150 μL
Mix thoroughly, place at 100℃ water bath for 10 minutes, cool to room temperature			
Distilled water	900 μL	900 μL	900 μL

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.