# Total Carbohydrate Content Assay kit

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

**Operation Equipment:** Spectrophotometer/Microplate reader

**Cat No:** BC2715

**Size:** 100T/96S

**Components:**

**Reagent I**: Liquid 100 mL×1 bottle, store at 2-8℃.

**Reagent II**: Liquid 100 mL×1 bottle, store at 2-8℃.

**Reagent III**: Liquid 5 mL×1 bottle, store at 2-8℃.

**Standard**: Power×1, 10mg of 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/microplate reader, centrifuge, water bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, 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 minutes, adjust the wavelength to 540nm, set the spectrophotometer counter to zero with distilled water.
2. Standard working solution: 10 mg/mL standard is diluted with distilled water to 1.6, 1.0, 0.8, 0.4, 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 | - | 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. 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.

**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 $\Delta A_T$ into the formula to get x (mg/mL).

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

1. Sample weight

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

2. Liquid volume

   Total Carbohydrate content (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. 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.
2. The degree of cellulose decomposition cannot reach 100% in this 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 $\Delta A_T=A_T-A_B$=0.599-0.05=0.549, standard curve y=0.8881x-0.0561, then x=0.6813. The result is calculated according to the sample weight:

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

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 $\Delta A_T=A_T-A_B$ = 0.594-0.05 = 0.544, standard curve y = 0.8881x-0.0561, then x = 0.6575. The result is calculated according to the sample weight:
   Total sugar content (mg/g weight) = 10×x÷W = 65.75 mg/g weight.
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, $\Delta A_T=A_T-A_B$ = 0.294-0.05 = 0.244, and the standard curve y = 0.8881x-0.0561, then x = 0.338. The result is calculated according to the sample volume:
   Total sugar content (mg/mL) = 10×x= 3.38 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: 0.0667 mg/mL
The linear range: 0.09-1.8 mg/mL