QuantiChrom™ Total Carbohydrate Assay Kit (TCRB-100)

Quantitative Colorimetric Total Carbohydrate Determination

DESCRIPTION

CARBOHYDRATES are made up of carbon, hydrogen, and oxygen. A carbohydrate may exist in its simplest form, a monosaccharide, or as larger polymers called polysaccharides (e.g. starch, glycogen, and cellulose). Carbohydrates are essential constituents of living things, and in the form of glucose, serve as the main source of energy for metabolism. They are also commonly found in many fruits and vegetables as simple or complex sugars.

BioAssay Systems' QuantiChrom™ Total Carbohydrate Assay Kit first hydrolyzes polysaccharides to monosaccharides, which are then converted to furfural compounds that react with the Detection Reagent. The color intensity of the chromagen, measured at 490 nm, is directly proportional to the total carbohydrate concentration (glucose equivalents) in the sample.

KEY FEATURES

Non-radioactive assay.

Sensitive and accurate. Linear detection range in 96-well plate: 0.02 to 5 mM glucose equivalents for colorimetric assays.

Fast and convenient. The procedure involves 15 minutes of incubation time and minimal sample preparation.

APPLICATIONS

For quantitative determination of total carbohydrate in food, beverage, biological samples (e.g. serum, plasma, etc).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

30X Detection Reagent: 220 µL **Standard:** 200 µL

Storage conditions. The kit is shipped at room temperature. Store all components at 4°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Beverage: For beverages that are carbonated, pour sufficient volume into a cup and shake gently at room temperature until no CO_2 bubbles are visible. If colored or turbid (i.e. contains pulp, etc.), centrifuge at 14,000 rpm for 5 minutes and transfer the clear supernatant to a clean tube. Many beverages require a steep dilution in dH_2O (i.e. 1000-fold) for the assay.

Serum or Plasma: Lithium heparin plasma may interfere with the assay and should be avoided if possible. Dilute 25-fold in dH_2O. If not assayed immediately, store at –80°C for up to 30 days.

For unknown samples, it is prudent to test several dilutions to determine the optimal sample dilution factor, *n*.

Reagent Preparation

Equilibrate all components to room temperature (25°C) prior to the assay. Briefly centrifuge tubes before use.

Procedure using 96-well plate

1. *Standards*. Prepare 80 µL 5 mM Premix by mixing 10 µL of the Standard (40 mM) with 70 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + dH_2O	D-Glucose (mM)
1	30 µL + 0 µL	5
2	18 µL + 12 µL	3
3	9 µL + 21 µL	1.5
4	0 µL + 30 µL	0

2. *Samples*. Transfer 30 µL of each sample to separate 1.5-mL centrifuge tubes.
3. Add 150 µL of concentrated H_2SO_4 (95%, not provided in kit) to each tube. Vortex and then briefly centrifuge.

   Caution: Handle H_2SO_4 with appropriate protective gear (i.e. fume hood, goggles, gloves, etc.).
4. Incubate at 90°C for 5 minutes on a heat block.
5. Remove tubes from 90°C and let cool for 5 minutes at 4°C.
6. Remove tubes from 4°C and briefly centrifuge.
7. Prepare sufficient 1X Detection Reagent by mixing for each tube, 2 µL of 30X Detection Reagent with 58 µL dH_2O.
8. Add 50 µL 1X Detection Reagent to each tube and briefly vortex on low power.
9. Incubate at room temperature for 5 min. Then, transfer 100 µL from each tube to separate wells in a clear, flat-bottom 96-well plate. Read OD at 490 nm (480-500 nm).

CALCULATION

Subtract the blank value (water, #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the Total Carbohydrate concentration (glucose equivalents) in the Sample as follows:

$$[\text{Total Carbohydrate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (mM^{-1})} \times n \quad (mM)$$

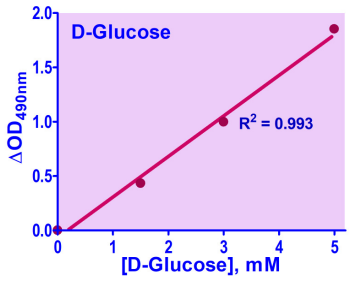
where OD_{SAMPLE} and OD_{BLANK} are the optical density values of the Sample and H_2O Blank, respectively. *n* is the sample dilution factor.

Note: If the calculated concentration is higher than 5 mM, dilute the sample in water and repeat the assay. Multiple the result by the dilution factor, *n*.

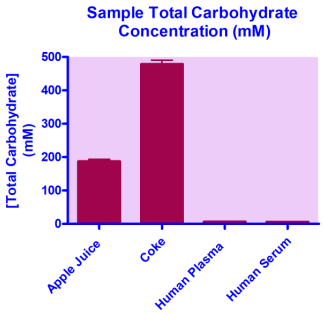
Unit conversion: 1 mM glucose is equiv. to 18 mg/dL or 180 ppm glucose.

MATERIALS REQUIRED, BUT NOT PROVIDED

Concentrated (95%) sulfuric acid (e.g. Rocky Mountain P/N S1068), heat block, pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and centrifuge, vortexer, and plate reader.



D-Glucose Standard Curve



Sample Total Carbohydrate Concentration (mM)

LITERATURE

1. Blanco, A. and Blanco, G. (2017). Carbohydrates. Medical Biochemistry. pp. 73-97.
2. Jéquier, E. (1994). Carbohydrates as a source of energy. The American Journal of Clinical Nutrition, 59(3), pp.682S685S.
3. Chinachoti, P. (1995). Carbohydrates: functionality in foods. The American Journal of Clinical Nutrition, 61(4), pp.922S929S.