Total Carbohydrate Assay Kit

Catalog No.: abx298986

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Total Carbohydrate concentrations in serum, plasma, tissue homogenates, cell lysates, food, juice, beverage, and other agricultural products.

Detection Range: 50 µg/ml – 500 µg/ml

Introduction: Carbohydrates are the most abundant macromolecules present in all living organisms. Many functions are played by carbohydrates: acting as structural components of cell walls in plants and bacteria, energy storage in the form of starch and glycogen. Carbohydrates are also a major dietary component of heterotrophs, including humans.

Abbexa's Total Carbohydrate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Carbohydrate concentrations. The dye reagents react with Carbohydrates to create an absorption maximum at 540 nm. The intensity of the color is proportional to the concentration of Carbohydrate, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer 1: 2 × 30 ml
3. Assay Buffer 2: 2 × 30 ml
4. Dye Reagent: 10 ml
5. Standard (0.5 mg/ml): 1 ml

Materials Required But Not Provided

1. Microplate reader (540 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Sonicator
7. Mortar
8. Water bath
9. Convection oven

Protocol

A. Preparation of Sample and Reagents

1. Sample

- **Tissue samples**

  Add 0.05 g of sample to a tube of 0.5 ml Assay Buffer 1 and homogenize. Heat in a boiling water bath for 30 minutes. Allow to cool to room temperature, and when cool, add 0.5 ml of Assay Buffer 2. Centrifuge at 8,000 × g at room temperature for 10 minutes. Transfer the supernatant to a new tube then analyze immediately.

- **Liquid samples**

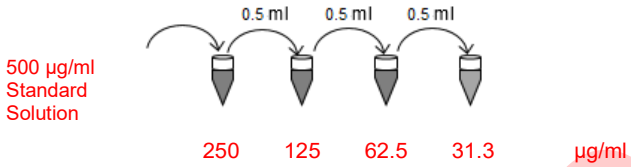
  Add 0.05 ml of sample to a tube of 0.5 ml Assay Buffer 1, and heat in a boiling water bath for 30 minutes. Allow to cool to room temperature, and when cool, add 0.5 ml of Assay Buffer 2. Centrifuge at 8,000 × g at room temperature for 10 minutes. Transfer the supernatant to a new tube then analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 4 tubes with 250 µg/ml, 125 µg/ml, 62.5 µg/ml, and 31.3 µg/ml. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 500 µg/ml Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube, mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 100 µl of sample to the sample wells.
4. Add 100 µl of prepared standards to the standard wells.
5. Add 100 µl of distilled water to the blank wells.
6. Add 100 µl of Dye Reagent to all wells.
7. Tap the plate gently to mix. Incubate in a convection oven at 90°C for 10 minutes.
8. Allow to cool to room temperature. When cool, read and record absorbance at 540 nm.

C. Calculations

Total Carbohydrate per g of sample:

$$\textbf{Total Carbohydrate (mg/g)} = 0.9 \times \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{W \times V_{Sample}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.45}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Total Carbohydrate per ml of liquid sample:

$$\textbf{Total Carbohydrate (mg/ml)} = 0.9 \times \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V \times V_{Sample}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.45}{V} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Standard}$	Concentration of highest standard (500 µg/ml = 0.5 mg/ml)
W	Weight of sample used in sample preparation (in g)
V	Volume of sample used in sample preparation (in ml)
V_{Assay}	Total volume of Assay Buffer 1 and Assay Buffer 2 used in sample preparation (1 ml)
V_{Sample}	Volume of sample used in the assay procedure (0.1 ml)
$V_{Standard}$	Volume of standard used in the assay procedure (0.1 ml)
0.9	Conversion factor