Total Carbohydrate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1201

(Version 1.2A)

Detection and Quantification of Total Carbohydrate Content

inSerum, Plasma, Tissue extracts, Cell lysate, Food, Juice, Beverage,

Other agricultural products Samples.

For research use only. Not for diagnostic or therapeutic procedures.

Cohesion BIOSCIENCES

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I. INTRODUCTION

Carbohydrates are the most abundant biomolecules present in all living organisms. Carbohydrates have many functions, as structural components to the cell walls of bacteria and plants, or as energy storage in the form of starch and glycogen. Carbohydrates are also amajor component of the human diet.

Total Carbohydrate Colorimetric Microplate Assay Kit can be used for measuring carbohydrates in a variety of samples, including food and beverage products. These compounds react with the developer to generate a chromagen, which can be detected at 540 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay BufferI	30 mlx 2	4 °C
Assay BufferII	30 mlx 2	4 °C
Dye Reagent	10 mlx 1	4 °C
Standard (0.5 mg/ml)	1 mlx 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8.Convection oven

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.05g samplein a centrifuge tube, homogenize with 0.5 ml Assay BufferI, put it in boiling water bath for 30 minutes,when cold, add 0.5 ml Assay BufferII;centrifuged at 8,000g at room temperature for 10minutes, take the supernatant into a new centrifuge tube for detection.

2.For liquid samples

Add 0.05mlsampleinto a centrifuge tube, then add0.5 ml Assay BufferI, put it in boiling water bath for 30 minutes,when cold, add 0.5 ml Assay BufferII; centrifuged at 8,000g at room temperature for 10minutes, take the supernatant into a new centrifuge tube for detection.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Dye Reagent	100µl	100µl	100µl
Mix, put the plate into the convection oven,90°C for 10minutes. When cold, record absorbance measured at 540 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

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

$=0.45 \times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$

2. According to the weight of sample

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

$= 0.45 \times(OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})/ W$

$C_{Standard}$: the standard concentration, 0.5mg/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

V: the volume of sample, ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

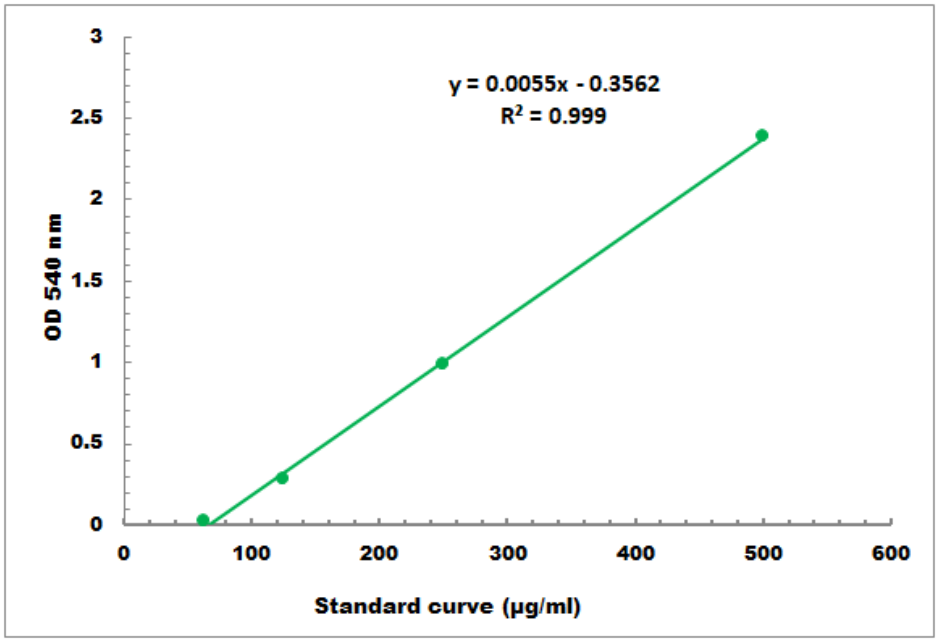
V_{Sample}: the volume of sample, 0.1 ml;

V_{Assay}: the volume of Assay BufferI and Assay BufferII, 1 ml;

0.9: conversion factor.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 50µg/ml - 500µg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES