Total Carbohydrate

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1201

(Version 1.3A)

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.

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, keep in dark
Standard (500 µg/ml)	1 mlx 1	4 °C
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Note: Avoid freeze-thaw cycles

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be500/250/125/62.5µg/ml.

V. 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.

VI. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu g/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu g/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu g/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, μg/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

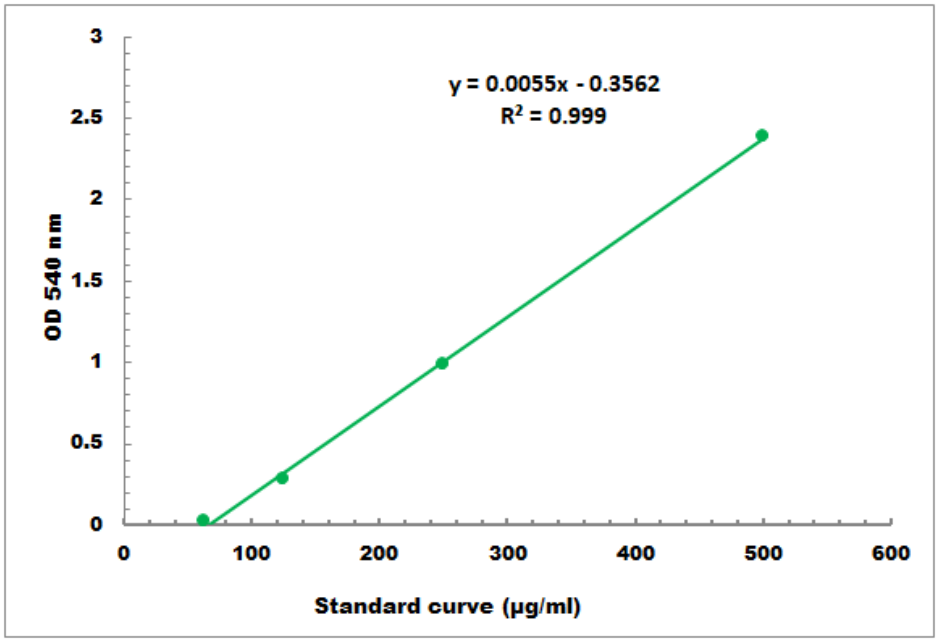
V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

V: the volume of sample in sample preparation, ml

VIII. TYPICAL DATA

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



Detection Range: 50µg/ml - 500µg/ml