Total Carbohydrate

Microplate Assay Kit

Cat #: orb707362 (manual)

Detection and Quantification of Total Carbohydrate Content in Serum, Plasma, Tissue extracts, Cell lysate, Food, Juice, Beverage, Other agricultural products Samples.

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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

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 a major component of the human diet.
Total Carbohydrate 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.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Dye Reagent	10 ml x 1	4 °C
Standard (0.5 mg/ml)	1 ml x 1	4 °C
Technical Manual	1 Manual	

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

SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.05 g sample in a centrifuge tube, homogenize with 0.5 ml Assay Buffer I, put it in boiling water bath for 30 minutes, when cold, add 0.5 ml Assay Buffer II; centrifuged at 8, 000g at room temperature for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

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

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put the plate into the convection oven, 90 °C for 10 minutes. 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.

CALCULATION

1. According to the volume of sample

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

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

2. According to the weight of sample

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

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

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

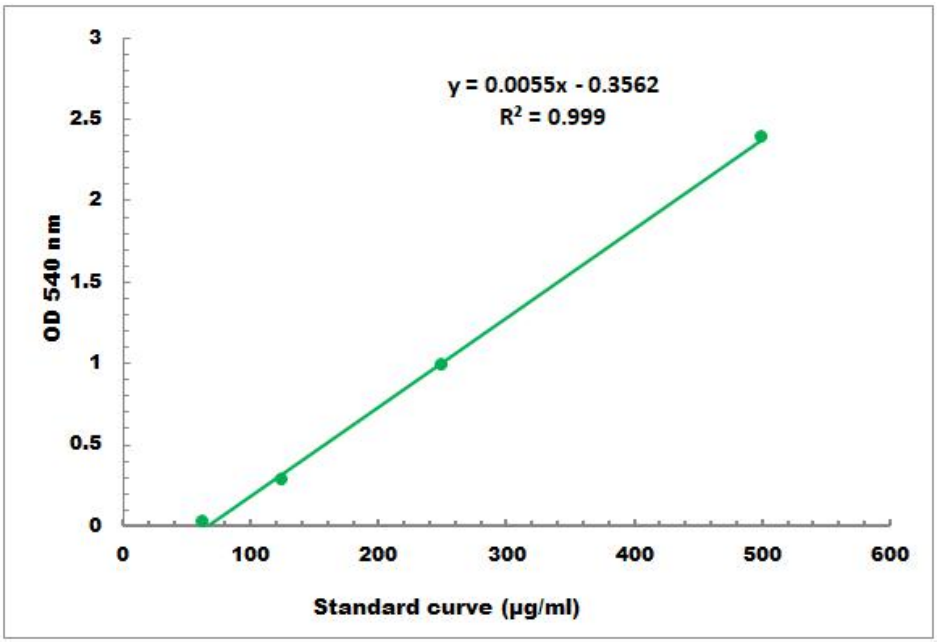
V_{Sample}: the volume of sample, 0.1 ml;

V_{Assay}: the volume of Assay Buffer I and Assay Buffer II, 1 ml;

0.9: conversion factor.

TYPICAL DATA

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



Detection Range: 50 µg/ml - 500 µg/ml