Total Carbohydrate Assay Kit

Cat #: orb1173188 (manual)

Size: 48 T/96 T

Product name: Total Carbohydrate Assay Kit

Catalog number: orb1173188

Sensitivity: 0.1 mg/mL

Detection range: 0.1-2 mg/mL

Applicable samples: Plant Tissues, Animal Tissues, Serum, Plasma

Storage: Stored at 4℃ for 12 months, protected from light

Assay Principle

Carbohydrate is one of the important components of animal and plant, and it is also the main raw material and storage substance of metabolism. Total carbohydrates mainly refer to reducing glucose, fructose, maltose, lactose, sucrose and maltose that can be hydrolyzed into reducing monosaccharides under the measurement conditions and starch that can possibly partially hydrolyzed. CheKine™ Micro Total Carbohydrate Assay Kit is specially developed for the detection of total carbohydrates in animal and plant tissues, serum (plasma) and other liquid samples. The total carbohydrates is hydrolyzed into reducing sugars. The reducing sugars are heated with DNS reagents under alkaline conditions to reduce DNS to generate amino compounds, which are reddish-brown in alkaline solution and have a characteristic absorption peak at 540 nm. Within a certain concentration range, the reducing sugar content has a linear relationship with the absorbance at 540 nm. According to the standard curve, the total Carbohydrates content in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Hydrochloric Acid	50 mL	100 mL	4℃
Sodium Hydroxide	50 mL	100 mL	4℃
DNS Reagent	2 mL	4 mL	4℃, protected from light
Standard	1	1	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, water bath

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

- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Hydrochloric Acid: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sodium Hydroxide: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

DNS Reagent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light. **Standard:** Before use, add 1 mL deionized water to the Standard powder and mix well to prepare 10 mg/mL Standard. Storage at 4℃.

Setting of standard curves: Dilute 10 mg/mL Standard to 2, 1.6, 1, 0.8, 0.4, 0.2, 0.1 mg/mL with deionized water.

Num.	Volume of 10 mg/mL Standard (µL)	Volume of Deionized Water (µL)	Concentration (mg/mL)
Std.1	40	160	2
Std.2	32	168	1.6
Std.3	20	180	1
Std.4	16	184	0.8
Std.5	8	192	0.4
Std.6	4	196	0.2
Std.7	2	198	0.1

Sample Preparation

1. Plant or animal tissue samples: Weigh 0.1 g tissue, add 1 mL Hydrochloric Acid, 1.5 mL deionized water, and homogenize, boiling water bath 30 min. Then add 1 mL Sodium Hydroxide, mix well, dilute with deionized water to 10 mL. Centrifuge at 8,000 g for 10 min at 25°C. Use supernatant for assay.
2. Serum or plasma sample: Take 0.1 mL plasma (serum), add 0.1 mL Hydrochloric Acid, 0.15 mL deionized water, and homogenize, boiling water bath 30 min. Then add 0.1 mL Sodium Hydroxide, mix well, dilute with distilled water to 1 mL. Centrifuge at 8,000 g for 10 min at 25°C. Use supernatant for assay.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each tube:

Reagent	Blank Tube (µL)	Standard Tube (µL)	Test Tube (µL)
Sample	0	0	30
Different Concentration Std.	0	30	0

Deionized Water	30	0	0
DNS Reagent	30	30	30

Mix well, boiling water bath 10 min (cover tightly to prevent water loss), take it out and cool to room temperature

Deionized Water	180	180	180

Mix well, take out 200 µL to a 96-well plate or microglass cuvette. Measure absorbance of 540 nm in a microplate reader, recorded as A_{Blank}, $A_{Standard}$ and A_{Test}, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the y axis and the $\Delta A_{Standard}$ as the x axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (mg/mL).

2. Calculating the content of total carbohydrate:

(1) By sample fresh weight

Total carbohydrates (mg/g)=$(y\times V_{Sample})\div(W\times V_{Sample}\div V_{Sample\ Total})\times n$**=10×y÷W×n**

(2) By protein concentration

Total carbohydrates (mg/mg prot)=$(y\times V_{Sample})\div(V_{Sample}\times Cpr)\times n$**=y÷Cpr×n**

(3) By liquid volume

Total carbohydrates (mg/mL)=$(y\times V_{Sample})\div(V_{Liquid}\times V_{Sample}\div V_{Sample\ Total})\times n$**=10×y×n**

Where: V_{Sample} is add sample volume, 0.03 mL; $V_{Sample\ Total}$ is total volume of tissue sample,10 mL; Cpr is the protein concentration of the sample, mg/mL; W is weight of sample, g; V_{Liquid} is liquid sample volume of be taken, 0.1 mL; $V_{Sample\ Total}$ is total volume of liquid sample, 1 mL; n is the sample dilution factor.

Typical Data

Typical standard curve:

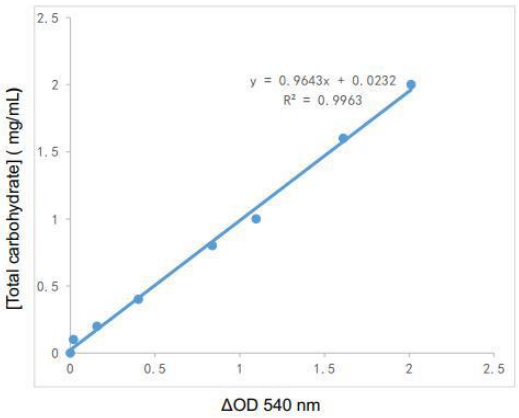

Figure 1. Standard curve for Total Carbohydrate.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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