Micro Total Carbohydrate Assay Kit

Catalogue No.: K139

Size: 96T

Range: 0.1-2mg/mL

Sensitivity: 0.1mg/mL

Kit component:

Reagents	96T	Storage
Hydrochloric Acid	120mL	2-8 °C
Sodium Hydroxide	120mL	2-8 °C
DNS Reagent	4.2mL	2-8 °C protected from light
Standard	Powder ×1 vial	2-8 °C

Storage:

The kit should be stored at 2-8℃ shading light for one year.

Application:

Detect the total Carbohydrate content in biological samples, including serum (or plasma), animal tissues, and plant tissues.

Principle of the Assay:

Carbohydrate substances are one of the important components that make up the bodies of animals and plants, and they are also the main raw materials and storage substances for metabolism. Total Carbohydrate mainly refers to reducing sugars such as glucose, fructose, and lactose, as well as sucrose, maltose and possibly partially hydrolyzed starch that can be hydrolyzed into reducing monosaccharides under certain measurement conditions.

The detection principle of this kit is as follows: total Carbohydrate is hydrolyzed into reducing sugars. These reducing sugars react with DNS reagent under alkaline conditions to reduce DNS and generate amino compounds. These compounds appear red-brown in alkaline solution and have a characteristic absorption peak at 540 nm. Within a certain concentration range, the content of reducing sugars has a linear relationship with the absorbance at 540 nm. Based on the standard curve, the total Carbohydrate content in the sample can be calculated.

Additional Materials Required:

Microplate reader or visible spectrophotometer (capable of measuring absorbance at 540nm), water bath, low-temperature centrifuge, 96-well plate or micro glass cuvette, adjustable pipette and pipette tips, homogenizer (for tissue samples), deionized water

Assay Procedure (For reference):

Reagent preparation

Hydrochloric Acid： Ready-to-use; Before use, balance to room temperature; Store at 4°C.

Sodium Hydroxide： Ready-to-use; Before use, balance to room temperature; Store at 4°C.

DNS Reagent： Ready-to-use; Before use, balance to room temperature; Store at 4°C in the dark.

10mg/mL Standard： Prepare immediately before use by adding 1 mL of deionized water to dissolve completely, resulting in a 10 mg/mL standard solution. Any unused dissolved standard should be transferred into a glass bottle, tightly sealed, and stored at 4°C away from light for up to one month.

Construction of the Standard Curve： Dilute 10 mg/mL Standard with deionized water to 2, 1.6, 1, 0.8, 0.4, 0.2, and 0.1 mg/mL. For each experiment, a standard sample test must be conducted to create a standard curve; the diluted standard solution is unstable and must be used within 4 hours.

Item	10 mg/mL Standard solution volume(μL)	The volume of deionized water (μL)	Standard 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
Blank	0	200	0

Note: For each experiment, please use new diluted standards.

Sample preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. During the measurement process, the temperature and time for thawing should be controlled. When thawing at room temperature, the samples should be completely thawed within 4 hours.

1. Organisms and tissues: Weigh approximately 0.1 g of the tissue, add 1 mL of Hydrochloric Acid, 1.5 mL of deionized water, homogenize, incubate in boiling water bath for 30 minutes, add 1 mL of Sodium Hydroxide, mix well, make up to 10 mL with deionized water, centrifuge at 8000 g for 10 minutes at 25℃, and take the supernatant for measurement.

2. Liquid samples (such as serum) : Take 0.1 mL of serum, add 0.1 mL of Hydrochloric Acid, 0.15 mL of deionized water, homogenize, heat in boiling water bath for 30 minutes, add 0.1 mL of Sodium Hydroxide, mix well, make up to 1 mL with deionized water, centrifuge at 8000 g for 10 minutes at 25℃, and take the supernatant for testing.

Experimental procedures

1.The microplate reader or visible spectrophotometer should be preheated for more than 30 minutes, and the wavelength should be adjusted to 540nm.
2. Sample determination (the blank wells and standard wells only need to be tested 1-2 times. Add the following reagents in sequence to a 1.5 mL ELP tube):

Reagent	Blank tube (μL)	Standard tube (μL)	Test tube (μL)
Sample	0	0	30
Different concentrations Std.	0	30	0
Deionized water	30	0	0
DNS Reagent	30	30	30
Mix well, place in boiling water bath for 10 minutes (cover tightly to prevent water loss), then remove and let it cool to room temperature			
Deionized water	180	180	180

3.Absorbance measurement: After mixing, take 200 μL from each tube and add it to the corresponding wells of a 96-well plate or a micro glass cuvette. Measure the absorbance at 540 nm, and calculate ΔA Test = A Test - A blank, ΔA standard = A standard - A blank.

Note: Before conducting the experiment, it is recommended to select 2-3 samples with significant expected differences for a preliminary test.

Calculation:

Note: The calculation formulas we provide for you include the derivation process formulas and the concise calculation formulas. Both are exactly the same. It is recommended to use the concise calculation formula in bold as the final calculation formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the ΔAStandard as the x-axis, draw the standard curve. Substitute ΔA into the standard curve formula to calculate y (mg/mL).

2. Calculation of total Carbohydrate content in the sample

(1) Calculated by protein concentration

Total sugar(mg/mg prot)=(y×V sample)÷(V sample×Cpr)×n=**y÷Cpr×n**

(2) Calculated by fresh weight of samples

Total sugar(mg/g)=(y×V sample)÷(W×V sample÷VS Sample Total)×n=**10×y÷W×n**

(3) Calculated by volume of Liquid samples

Total sugar(mg/mL)=y×V sample÷(V Liquid×V sample÷VL Sample Total)×n=**10×y×n**

Note:

V sample: Add sample volume, 0.03 mL;

VS Sample Total: Total volume of the sample, 10 mL;

Cpr: Sample protein concentration, mg/mL;

W: weight of sample, g;

V Liquid: Volume of the liquid sample, 0.1 mL;

VL Sample Total: Total volume of the liquid sample, 1 mL;

n: Dilution factor.

Notes:

1.For tissue samples and cell samples, the results can be normalized between samples by measuring protein concentration. It is recommended to use the protein quantitative kit K001 (BCA method) from FineTest.

2.This kit is compatible with spectrophotometer detection. Please adjust the amount of test reagents according to the requirements of spectrophotometer detection in proportion.

3.It is recommended that the experimenters establish their own standard curves to improve the accuracy of the results. If they do not establish their own standard curves, they can use the typical standard curve formulas presented in the result display section for calculation.

4.This product is only limited to scientific research personnel.

5.Please pay attention to safety precautions and follow the operating procedures for laboratory reagents. For your safety and health, please wear a lab coat and use disposable gloves during the operation.