（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K755-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (530-550 nm)

Detection range：0.036-1.0 mg/mL

Elabscience® Total Carbohydrate Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

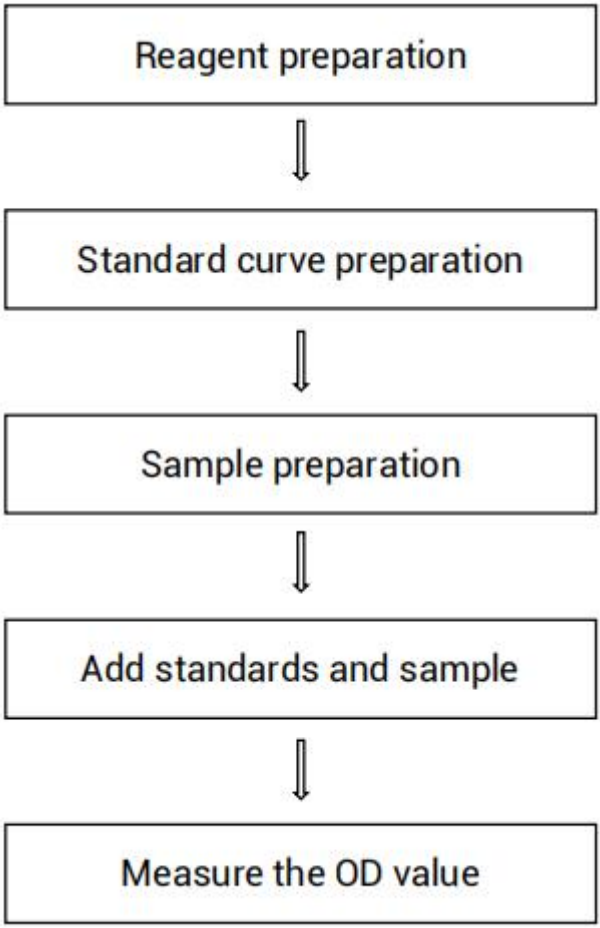
Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can measure total carbohydrate content in serum, plasma, animal and plant tissue samples.

Detection principle

Carbohydrate is one of the important components of various bodies, and is also the main raw material and storage material of metabolism. Total carbohydrate mainly refers to reducing glucose, fructose, pentose, lactose, maltose, sucrose that can be hydrolyzed to reducing monosaccharides under determined conditions, and possibly partially hydrolyzed starch. The total carbohydrate was hydrolyzed to reducing sugar, which was reduced to amino compounds after co-heating with 3, 5-dinitrosalicylic acid under alkaline conditions. The amount of reducing sugar was proportional to the color of the reddish brown substance, and the total carbohydrate content in the sample was determined by this method.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Extraction Solution A	30 mL × 1 vial	55 mL × 1 vial	2-8°C, 12 months
Reagent 2	Extraction Solution B	30 mL × 1 vial	55 mL × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	2 mL × 1 vial	4 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Standard	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (530-550 nm, optimum wavelength: 540 nm), Water bath

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② Before testing, incubate the chromogenic agent in 75℃ water bath, then cool it to 25℃ with running water.

③ The preparation of 10 mg/mL standard solution:
Dissolve one vial of standard with 1 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 1 month.

④ The preparation of 1 mg/mL standard solution:
Before testing, please prepare sufficient 1 mg/mL standard solution. For example, prepare 1000 μL of 1 mg/mL standard solution (mix well 100 μL of 10 mg/mL standard solution and 900 μL of double distilled water). Store at 2-8°C for 2 weeks.

⑤ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 1 mg/mL standard solution with double distilled water to a

serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
1 mg/mL Standard (μL)	0	20	40	60	80	120	160	200
Double distilled water (μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation

Serum or plasma samples:

① Take 0.1 mL of serum (plasma) to 2 mL tube. Add 0.1 mL of extraction solution A and 0.15 mL of double distilled water into the tube.

② Mix well, and incubate the tubes in 95℃ water bath for 30 min. Cool the tubes to 25℃ with running water.

③ Add 0.1 mL of extraction solution B, mix well.

④ Centrifuge at 8000×g for 10 min at 25℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation
50 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Take 50 mg of tissue to 2 mL tube. Add 0.5 mL of extraction solution A and 0.75 mL of double distilled water into the tube with a dounce homogenizer at 4℃.

④ Incubate in 95°C water bath for 30 min.

⑤ Add 0.5 mL of extraction solution B, mix well.

⑥ Centrifuge at 8000×g for 10 min at 25℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Wheat tissue homogenate	30-60
10% Paddy tissue homogenate	30-60
10% Corn tissue homogenate	30-60
10% Pumpkin tissue homogenate	30-50
10% Mouse liver tissue homogenate	3-10
Human serum	1

Note: The diluent is extraction solution A. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

The decomposition degree of cellulose in this kit cannot reach 100%.

Operating steps

① Standard tube: Add 30 μL of standard with different concentrations to the tubes.
Sample tube: Add 30 μL of sample to the tubes.

② Add 30 μL of chromogenic agent to each tube.

③ Mix well. Incubate the tubes in 95℃ water bath for 30 min, and cool it to 25℃ with running water.

④ Add 180 μL of double distilled water to each tube.

⑤ Mix fully and take 200 μL of reaction solution to the microplate.
Measure the OD value of each well at 540 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum and plasma samples:

$$\text{total carbohydrate content (mg/mL)} = \frac{\Delta A - b}{a} \times V_2 \div V_3 \times f$$

2. Tissue sample:

$$\text{total carbohydrate content (mg/g wet weight)} = \frac{\Delta A - b}{a} \times V_1 \div m \times f$$

[Note]

ΔA: $\Delta A = A_{sample} - A_{blank}$.

V_1: Total volume of tissue sample after treatment, 1.75 mL

m: The wet weight of sample, 0.05 g.

V_2: Total volume of serum (plasma) or liquid sample after treatment, 0.45 mL.

V_3: The volume of serum (plasma) or liquid sample, 0.1 mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.5	0.7	0.9
%CV	1.0	1.9	2.9

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/mL)	0.25	0.50	0.75
%CV	2.1	4.5	5.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/mL)	0.5	0.7	0.9
Observed Conc. (mg/mL)	0.55	0.74	0.93
Recovery rate (%)	105.0	106.0	103.0

Sensitivity

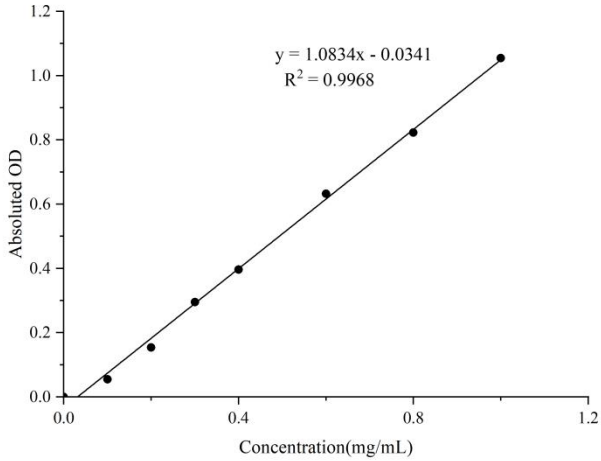
The analytical sensitivity of the assay is 0.036 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mg/mL)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
OD Value	0.055	0.109	0.215	0.352	0.458	0.675	0.882	1.104
	0.055	0.111	0.203	0.349	0.444	0.700	0.874	1.115
Average OD	0.055	0.110	0.209	0.351	0.451	0.688	0.878	1.110
Absoluted OD	0.000	0.055	0.154	0.296	0.396	0.633	0.823	1.055



Appendix Π Example Analysis

Example analysis：

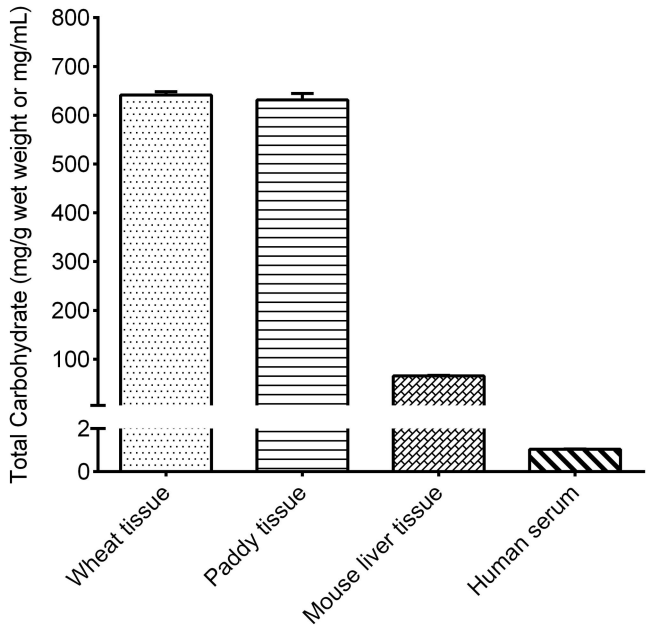
Take 30 μL of 10% wheat tissue homogenate which dilute for 50 times, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.0834 x - 0.0341, The OD value of the sample well is 0.418, the OD value of the blank well is 0.055, and the calculation result is:

total carbohydrate content (mg/g wet weight) = (0.418 - 0.055 + 0.0341) ÷ 1.0834

× 1.75 ÷ 0.05 × 50 = 641.43 mg/g wet weight

Detect 10% wheat tissue homogenate (dilute for 50 times), 10% paddy tissue homogenate (dilute for 50 times), 10% mouse liver tissue homogenate (dilute for 10 times) and human serum, according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.