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

Catalog No：E-BC-K866-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader（610-630 nm）

Detection range：0.003-1.5 mg/mL

Elabscience®Plant Soluble Sugar Colorimetric Assay Kit

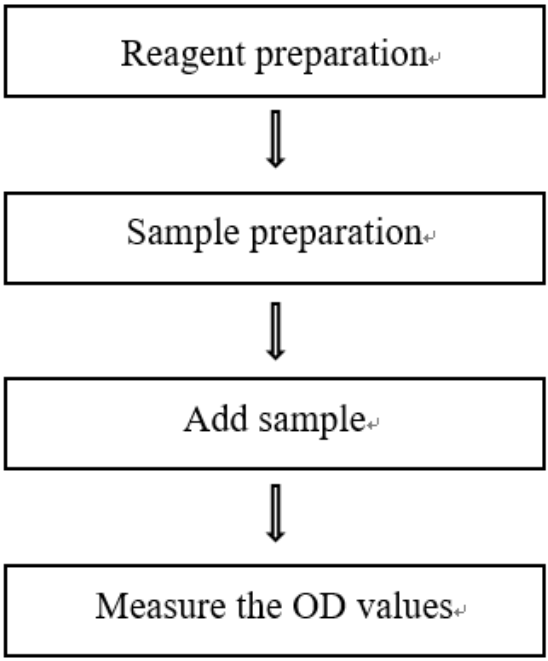
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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 soluble sugar content in plant sample.

Detection principle

Carbohydrate is one of the important components of plant corpus, and is also the main raw material of metabolism and storage material. The kit is used for the determination of soluble monosaccharides, oligosaccharides and polysaccharides. It has the advantages of high sensitivity, quick convenience and suitable for the determination of microscale samples. The detection principle is anthrone colorimetry. Carbohydrate react with anthrone to produce colored material, which has the maximum absorption peak at 620 nm. The soluble sugar content can be determined by measuring the absorbance value.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Substrate	Power ×1 vial	Power ×2 vials	2-8°C, 12 months, shading light
Reagent 2	1 mg/mL Standard	1 mL ×1 vial	1 mL ×2 vials	2-8°C, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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 (610-630 nm, optimum wavelength: 620 nm)

Reagents:

Concentrated sulfuric acid, Ethyl acetate

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：

Dissolve one vial of substrate with 6 mL of ethyl acetate at 60℃ water bath for 1-2 min well. Store at 2-8 ℃ for 7 days protected from light.

③ Preparation of 0.1 mg/mL standard：

For each well, prepare 200 μL of 0.1 mg/mL standard (mix well 20 μL of 1 mg/mL standard and 180 μL of double distilled water). Store at 2-8 ℃ and the prepared solution should be used up within 3 days.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

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

③ Homogenize 30 mg tissue in 270 μL double distilled water with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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% Mango tissue homogenate	150-250
10% Fresh jujube tissue homogenate	150-250
10% Grape tissue homogenate	150-200
10% Corn tissue homogenate	100-200
10% Apple tissue homogenate	100-150
10% Banana tissue homogenate	150-200
10% Cucumber tissue homogenate	20-50
10% Tomato tissue homogenate	30-60
10% Mushroom tissue homogenate	1
10% Carrot tissue homogenate	40-60

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The preparation of substrate working solution should be incubated at 60 ℃ water bath for 1-2 min until to be clarify.

② The heat release in the process of adding concentrated sulfuric acid may make the liquid splash, and add the sample slowly by sticking to the tube wall.

③ It is recommended to add the sample in the fume hood as far as possible.

④ The concentration of sulfuric acid must be over 95%. Concentrated sulfuric acid may absorb water during long term storage, and may interfere the results.

Operating steps

① Blank tube: Add 0.2 mL of double distilled water to the 2 mL EP tube.
Standard tube: Add 0.2 mL of 0.1 mg/mL standard to the 2 mL EP tube.
Sample tube: Add 0.2 mL of sample to the 2 mL EP tube.

② Add 0.1 mL of substrate working solution and 1 mL of concentrated sulfuric acid to each tube.

③ Mix fully, and incubate the tubes at 95-100℃ for 7 min (Fasten the tube mouth when heating), then cool the tubes to with running water immediately.

④ Take 200 μL the reaction solution of each tube to the microplate with a micropipette. Measure the OD value of each well at 620 nm with microplate reader.

Calculation

The sample:

$$\text{Soluble sugar content (mg/g wet weight)} = \frac{A_{Sample} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \div \frac{W}{V} \times f$$

[Note]

$A_{Satandrd}$: The OD value of standard well.

A_{Blank}: The OD value of blank well.

A_{Sample}: The OD value of sample well.

$C_{Standard}$: The concentration of standard, 0.1 mg/mL.

m: The weight of the sample, g.

V: The volume of homogenate, mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three banana tissue 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）	0.09	0.53	1.20
%CV	2.2	1.7	1.2

Inter-assay Precision

Three banana tissue 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）	0.09	0.53	1.20
%CV	5.1	4.9	5.9

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （mg/mL）	0.06	1.05	1.4
Observed Conc. （mg/mL）	0.1	1.1	1.4
Recovery rate(%)	103	102	101

Sensitivity

The analytical sensitivity of the assay is 0.003 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.

Appendix II Example Analysis

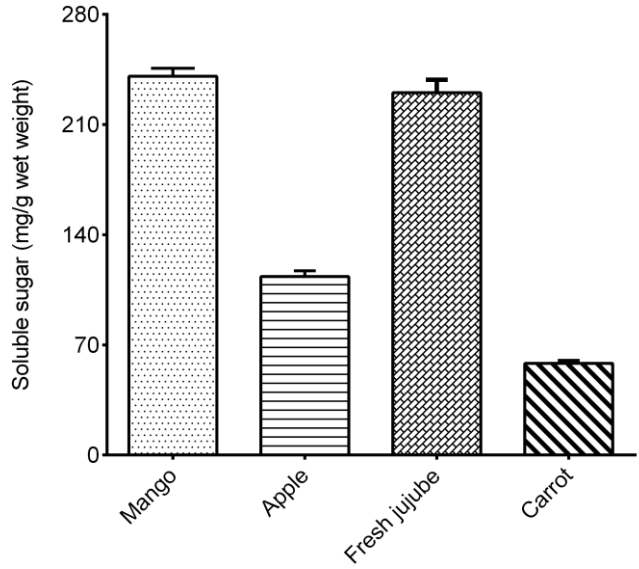
Example analysis:

For 10% mango tissue homogenate, dilute for 200 times, then take 200 uL sample and carry the assay according to the operation steps. The results are as follows:

The value of the blank well is 0.068, the value of the standard well is 0.375, the value of the sample well is 0.478, and the calculation result is:

Soluble sugar content (mg/g wet weight) = (0.478 – 0.068) ÷ (0.375 – 0.068) × 0.1 ÷ (0.1 ÷ 0.9) ×200 = 240.39 mg/g wet weight

Detect 10% mango tissue homogenate (dilute for 200 times), 10% apple tissue homogenate (dilute for 100 times), 10% fresh jujube tissue homogenate (dilute for 200 times), and 10% carrot tissue homogenate (dilute for 50 times) 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.