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

Catalog No：E-BC-K161-S

Specification：50 Assays(48 samples)/100 Assays(96 samples)

Measuring instrument：Spectrophotometer（290 nm）

Detection range：0.32-70 μmol/mL

Elabscience® Sucrose 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.

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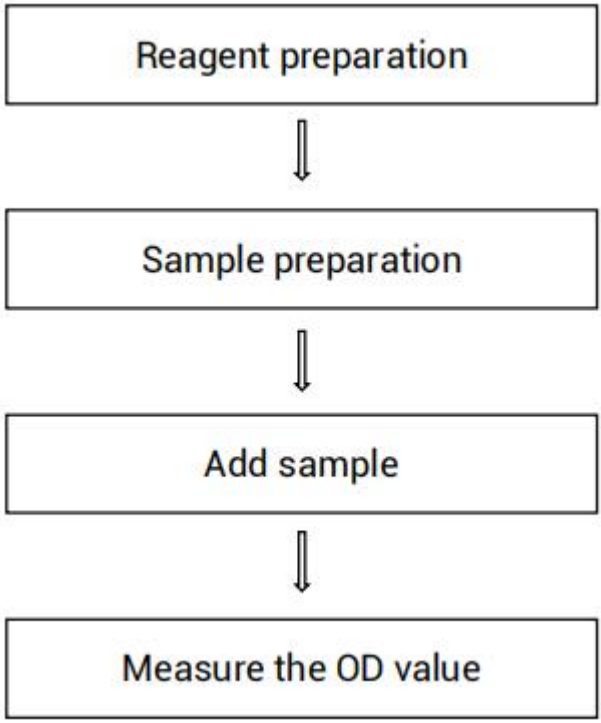
Assay summary

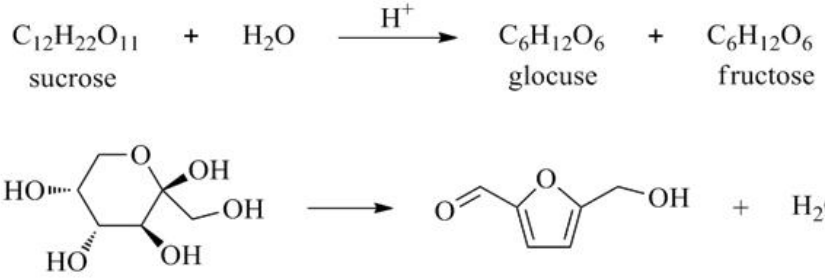


Intended use

This kit can be used to measure sucrose content in plant tissue samples.

Detection principle

Sucrose in plant tissue is hydrolyzed to glucose and fructose in boiling water bath under acidic conditions. 5-hydroxymethyl furfural was synthesized from fructose under acid condition and measure the ultraviolet absorption of 5-hydroxymethyl furfural. Glucose must be dissimilated into ketose structure and reduced to obtain 5-hydroxymethylfurfural, but the rate of isomerization of glucose to ketose is very slow. Therefore, the ultraviolet absorption of glucose is



much smaller than fructose.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Hydrolysate Solution	60 mL × 2 vials	60 mL × 4 vials	2-8°C, 12 months
Reagent 2	100 μmol/mL Sucrose Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months

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:

Spectrophotometer (290 nm), Micropipettor, Vortex mixer, 100°C Water bath.

Reagents:

Double distilled water, normal saline (0.9% NaCl)，PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of 20 μmol/mL sucrose standard：

For each well, prepare 30 μL of 20 μmol/mL sucrose standard (mix well 6 μL of 100 μmol/mL sucrose standard and 24 μL of double distilled water). The 20 μmol/mL sucrose standard should be prepared on spot. Store at 4°C for 7 days.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

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

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 3100×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
10% Green pepper tissue homogenization	1
10% Epipremnum aureum tissue homogenization	1
10% Cucumber tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The temperature of the water bath must be stable above 95°C.

② Glass tubes must be used in this experiment.

③ The detection of OD value should be completed within 20 min.

Operating steps

① Blank tube: add 0.03 mL of double distilled water into a 5 mL glass tube.
Standard tube: add 0.03 mL of 20 μmol/mL sucrose standard into a 5 mL glass tube.
Sample tube: add 0.03 mL of sample into a 5 mL glass tube.
② Add 2.0 mL of hydrolysate solution and mix fully with a vortex mixer.
③ Tighten the tubes with preservative film and make a hole on the film. Incubate the tubes in 100°C water bath for 8 min. Cool the tubes with running water.
④ Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 290 nm with 1 cm optical path quartz cuvette.

Calculation

The sample:

Tissue sample:

$$\text{Sucrose concentration (μmol/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 20 μmol/mL

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three cucumber 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 (μmol/mL)	8.50	34.80	58.00
%CV	3.7	3.4	3.1

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/mL)	8.50	34.80	58.00
%CV	8.3	8.1	9.1

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/mL)	12.6	45.5	60
Observed Conc. (μmol/mL)	13.4	44.1	61.8
Recovery rate (%)	106	97	103

Sensitivity

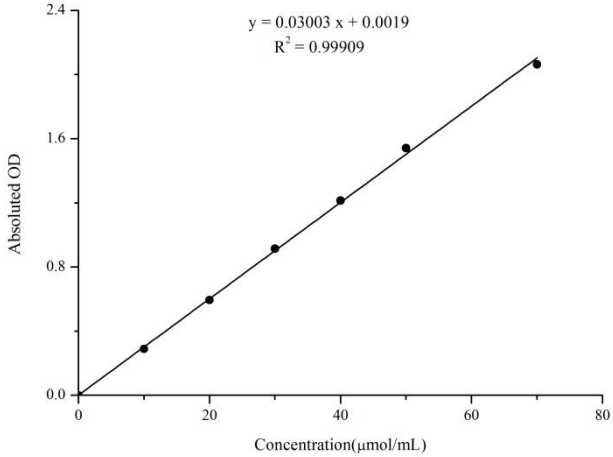
The analytical sensitivity of the assay is 0.32 μmol/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (μmol/mL)	0	10	20	30	40	50	70
Average OD	0.007	0.296	0.600	0.921	1.220	1.547	2.071
Absoluted OD	0	0.289	0.593	0.914	1.213	1.540	2.064



Appendix Π Example Analysis

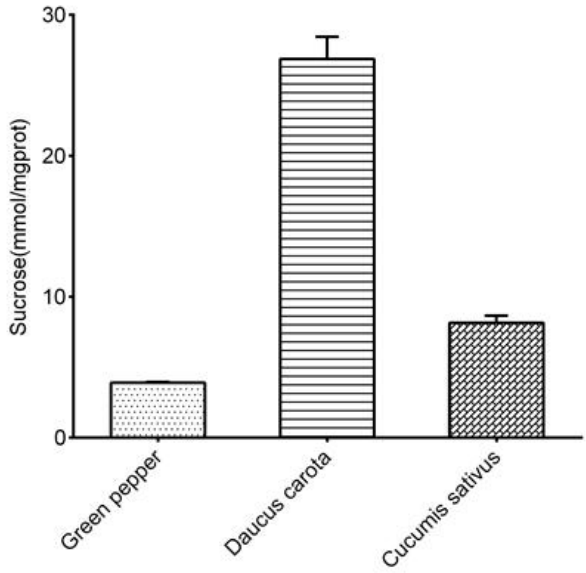
Example analysis：

Take 0.03 mL of 10% green pepper tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.229, the average OD value of the blank is 0.001, the average OD value of the standard is 0.675, the concentration of protein in sample is 1.73 mgprot/mL, and the calculation result is:

$$\text{Sucrose concentration (μmol/mgprot)} = \frac{0.229 - 0.001}{0.675 - 0.001} \times 20 \div 1.73 = 3.91\ (\mu mol/gprot)$$

Detect 10% green pepper tissue homogenate (the concentration of protein in sample is 1.73 mgprot/mL), 10% daucus carota tissue homogenate (the concentration of protein in sample is 0.74 mgprot/mL), 10% cucumis sativus tissue homogenate (the concentration of protein in sample is 0.69 mgprot/mL) 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.