PRODUCT INFORMATION & MANUAL

Sucrose Assay Kit (Colorimetric)

NBP3-25806

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Sucrose Colorimetric Assay Kit

Catalog No: NBP3-25806

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.32 μmol/mL

Detection range: 0.32-70 μmol/mL

Average intra-assay CV (%): 3.4

Average inter-assay CV (%): 8.5

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

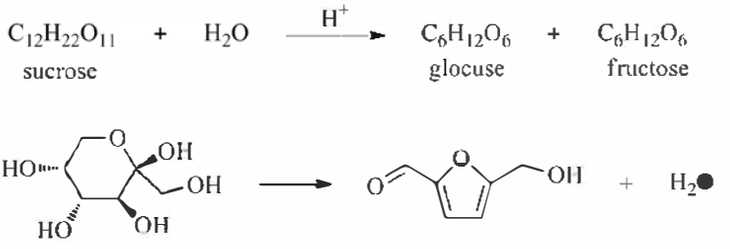
This kit can be used to measure sucrose content in plant tissue samples.

▲Background

Sucrose is a disaccharide which composed by glucose and fructose and it is the main nutrient in most plant cells. The biosynthesis of sucrose depends on the catalysis of sucrose phosphate synthase and 6'-sucrose phosphate phosphatase. It can participate in glycolysis and tricarboxylic acid cycle to produce ATP and NADH.

▲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	Specification	Storage
Reagent 1	Hydrolysate Solution	60 mL × 4 vials	2-8℃ , 12 months
Reagent 2	100 μmol/mL Sucrose Standard	1 mL × 1 vial	2-8℃ , 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.			

▲Materials prepared by users

Instruments

Spectrophotometer (290 nm), Micropipettor, Vortex mixer, 100℃ Water bath

Reagents

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The temperature of the water bath must be stable above 95℃ .
2. Glass tubes must be used in this experiment.
3. The detection of OD value should be completed within 20 min.

Pre-assay preparation

▲Reagent preparation

Preparation of 20 μmol/mL sucrose standard

Dilute the reagent 2 with double distilled water at a ratio of 1:4. Prepare the fresh solution before use. Prepared solution can be stored at 4℃ for 7 days.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.32-70 μmol/mL).

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).

Assay protocol

▲Detailed 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 reagent 1 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℃ 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.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.03		
20 μmol/mL sucrose standard (mL)		0.03	
Sample (mL)			0.03
Reagent 1 (mL)	2	2	2
Mix fully. Tighten the tubes with preservative film and make a hole on the film. Incubate the tubes in 100℃ water bath for 8 min. Cool the tubes with running water. Set the spectrophotometer to zero with double-distilled water and measure the OD values at 290 nm.			

▲Calculation

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

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 Data

▲Example analysis

Take 0.03 mL of 10% green pepper tissue homogenate, and carry the assay according to the operation table. 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:

Sucrose concentration(μmol/mgprot)=(0.229-0.001)÷(0.675-0.001)×20÷1.73
= 3.91 (μmol/mgprot)

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 3100 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M, E-BC-K168-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

Homogenized method:

1. Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

2. Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

Appendix III References

1. Winter H, Huber S C. Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes[J]. Crc Critical Reviews in Biochemistry, 2000, 35(4): 253-289.