PRODUCT INFORMATION & MANUAL

Sucrose Assay Kit (Fluorometric)

NBP3-25807

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

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Sucrose Fluorometric Assay Kit

Catalog No: NBP3-25807

Method: Fluorimetric method

Specification: 96T (Can detect 39 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.15 μmol/L

Detection range: 0.15-15 μmol/L

Average intra-assay CV (%): 2.3

Average inter-assay CV (%): 6.5

Average recovery rate (%): 96

- ▲ 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, a kind of disaccharide, is a major component of sugar. It is widely found in the leaves, flowers, stems, seeds and fruits of plants. It is an important product of plant photosynthesis and a major form of storage, accumulation and transportation of sugar in plants. Sucrose biosynthesis is catalyzed by sucrose phosphate synthase and 6 '-sucrose phosphate phosphatase and can through glycolysis and tricarboxylic acid cycle to produce ATP and NADH. Sucrose is particularly rich in sugarcane, sugar beet and other sugar crops and fruits. It is an important condiment of food and sweet.

▲Detection principle

Sucrose can be hydrolyzed by sucrase to produce glucose under acidic conditions, which is catalyzed by glucose oxidase to produce hydrogen peroxide. In the presence of HRP (horse radish peroxidase), hydrogen peroxide reacts with the fluorescent probe to form red fluorescent substance. The sucrose content can be calculated by measuring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution	45 mL × 1 vial	-20℃ , 12 months
Reagent 2	Enzyme Reagent A	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Buffer Solution	10 mL × 1 vial	-20℃ , 12 months
Reagent 4	Enzyme Reagent B	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 5	Probe	0.25 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	-20℃ , 12 months
	Black 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.

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/590 nm), Micropipettor, Incubator, Water bath, Centrifuge

⚠ 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 volume of reagent 2 must be strictly controlled, otherwise it will produce a large error.
2. Fluorescent probe reaction must be with shading light.

Pre-assay preparation

▲Reagent preparation

1.Bring all reagents to room temperature before use.

2.The preparation of reagent 1 working solution:

Mix the reagent 1 and double distilled water at a ratio of 1:9 fully. Prepare the fresh solution before use. The prepared solution can be stored at 2-8℃ for 7 days.

3.The preparation of reagent 2 working solution:

Dissolve a vial of reagent 2 with 300 μL of double distilled water and mix fully. The prepared solution can be stored at -20℃ for 7 days with shading light.

4.The preparation of reagent 4 working solution:

Dissolve a vial of reagent 4 with 0.25 mL of double distilled water and mix fully. The prepared solution can be stored at -20℃ for 7 days with shading light.

5.The preparation of reaction working solution:

Mix the reagent 3, reagent 4 working solution and reagent 5 at a ratio of 46:2:2 fully. Prepare the fresh solution before use.

6.The preparation of 10 mmol/L standard:

Dissolve a vial of reagent 6 with 10 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8℃ for 7 days.

The preparation of 100 μmol/L standard:

Dilute 10 μL of 10 mmol/L standard fully with 990 μL of reagent 1 working solution at a ratio of 1:99. Prepare the fresh solution before use.

▲Sample preparation

Tissue sample:

Weigh o1 g tissue accurately. Add 0.9 mL of reagent 1 working solution in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break tissue fully. Then centrifuge at 12000 g for 10 min at 4℃ and collect the supernatant for measurement. If not detected on the same day, the supernatant can be stored at -20℃ for 5 days.

▲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 (0.15-15 μmol/L).

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

Sample type	Dilution factor
10% Corn tissue homogenate	1500
10% Potato tissue homogenate	300-500
10% Tomato tissue homogenate	200-300
10% Macrophanerophytes leaf tissue homogenate	200-400
10% Carrot tissue homogenate	1500
10% Onion tissue homogenate	500-1000
10% Green pepper tissue homogenate	500-1000
10% Bush leaves tissue homogenate	20-50

Note: The diluent is reagent 1 working solution.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1’	S9	S9’	S17	S17’	S25	S25’	S33	S33’
B	B	B	S2	S2’	S10	S10’	S18	S18’	S26	S26’	S34	S34’
C	C	C	S3	S3’	S11	S11’	S19	S19’	S27	S27’	S35	S35’
D	D	D	S4	S4’	S12	S12’	S20	S20’	S28	S28’	S36	S36’
E	E	E	S5	S5’	S13	S13’	S21	S21’	S29	S29’	S37	S37’
F	F	F	S6	S6’	S14	S14’	S22	S22’	S30	S30’	S38	S38’
G	G	G	S7	S7’	S15	S15’	S23	S23’	S31	S31’	S39	S39’
H	H	H	S8	S8’	S16	S16’	S24	S24’	S32	S32’	X	X

[Note]: A-H, standard wells; X, blank wells; S1-S39, sample wells; S1’-S39’, control wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 100 μmo/L glucose standard with reagent 1 working solution to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 5, 8, 10, 12, 15 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	100 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	500
B	1	5	495
C	2	10	490
D	5	25	475
E	8	40	460
F	10	50	450
G	12	60	440
H	15	75	425

The measurement of samples

1) Standard well: add 2.5 μL of reagent 2 working solution into the corresponding wells.
   Sample well: add 2.5 μL of reagent 2 working solution into the corresponding wells..
   Control well: add 2.5 μL of reagent 1 working solution into the corresponding wells
   Blank well: add 2.5 μL of reagent 1 working solution into the corresponding wells.
2) Add 50 μL of standards with different concentrations into the standard wells.
   Add 50 μL of sample into the sample and control wells.
   Add 50 μL of reagent 1 working solution into the blank wells.
3) Mix fully with microplate reader for 10 s and incubate at 37℃ for 15 min.
4) Add 50 μL of reaction working solution into each well.
5) Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm.

▲Operation table

	Standard well	Sample well	Control well	Blank well
Reagent 2 working solution (μL)	2.5	2.5		
Reagent 1 working solution (μL)			2.5	2.5
Standards with different concentrations (μL)	50			
Sample (μL)		50	50	
Reagent 1 working solution (μL)				50
Mix fully and incubate at 37℃ for 15 min.				
Reaction working solution (μL)	50	50	50	50
Mix fully and incubate at 37℃ for 30 min.Measure the fluorescence intensity of each well.				

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample.

The standard curve is: y= ax + b.

Sucrose content (μmo/g wet weight)= (ΔF- b) ÷a× V× f÷W÷1000

Note:

y: $F_{Standard} - F_0$. (F_0 is the fluorescence value when the standard concentration is 0)
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve;
ΔF: Absoluted fluorescence intensity of sample, $\Delta F = (F - F_0) - (F' - F_0')$;
F: The fluorescence intensity of sample well;
F': The fluorescence intensity of control well;
F_0': The fluorescence intensity of blamk well;
V: The total volume of tissue extraction, 0.9 mL;
f: Dilution factor of sample before test;
W: The weight of plant tissue, 0.1 g;
10^3: The coefficient of unit conversion.

Appendix I Data

▲Example analysis

For 10% corn grain tissue homogenate, take 2.5 μL of sample supernatant diluted for 1500 times and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 1044.1 x + 12.953, the average of F is 10950, the average of F' is 1215, the average of F_0 is 3075, the average of F_0' is 957, ΔF = (10950 - 3075) – (1215 – 957) = 7617, and the calculation result is:

Sucrose content (μmol/g wet weight)

= (7617-12.953) ÷1044.1×0.9× 1500÷0.1÷1000

=98.32 μmo/g wet weight

Appendix II References

1. Dictionary of Nutrition Science. 2013(9): 603.
2. Paul C. Validation of a Simple, Colorimetric, Microplate Assay Using Amplex Red for the Determination of Glucose and Sucrose in Potato Tubers and Other Vegetables. Potato Association of America, 2008(85): 414-421.
3. Winter H. Regulation of Sucrose Metabolism in Higher Plants: Localization and Regulation of Activity of Key Enzymes. Crc Critical Reviews in Biochemistry, 2000,35(4): 253-289.