PRODUCT INFORMATION & MANUAL

Plant Soluble Sugar Assay Kit (Colorimetric)

NBP3-25922

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

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Plant Soluble Sugar Assay Kit (Colorimetric)

Catalog No: NBP3-25922

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.003 mg/mL

Detection range: 0.003-1.5 mg/mL

Average intra-assay CV (%): 1.7

Average inter-assay CV (%): 5.3

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 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	Specification	Storage
Reagent 1	Substrate	Power × 2 vials	2-8°C, 12 months, shading light
Reagent 2	1 mg/mL Standard	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.			

▲Materials prepared by users

Instruments:

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

Reagents:

Concentrated sulfuric acid, Ethyl acetate

⚠ 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 preparation of reagent 1 working solution should be incubated at 60°C 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.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 with 6 mL of ethyl acetate at 60°C water bath for 1-2 min fully. The prepared solution can be stored at 2-8°C with shading light for 7 days.
3. Preparation of 0.1 mg/mL standard:
   Mix the reagent 2 and double distilled water at the ratio of 1:9 fully. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 3 days.

▲Sample preparation

Tissue sample:

Accurately weigh the tissue, add double distilled water at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample on ice. Then centrifuge at 10000 g for 10 min, then take the supernatant for detection.

▲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 (0.003-1.5 mg/mL).

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.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank wells; B, standard wells; S1-S92, sample wells.

▲Detailed operation 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/mLstandard to the 2 mL EP tube.

   Sample well: Add 0.2 mL of sample to the 2 mL EP tube.

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

3) Mix fully, and incubate the tubes at 95-100°C 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.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.2		
0.1 mg/mLStandard (mL)		0.2	
Sample (mL)			0.2
Reagent 1 working solution (mL)	0.1	0.1	0.1
Concentrated sulfuric acid (mL)	1.0	1.0	1.0
Mix fully, and incubate the tubes at 95-100°C for 7 min (Fasten the tube mouth when heating), then cool the tubes to with running water immediately.			
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

Soluble sugar content(mg/g wet weight)

$$= (A_{Sample} - A_{Blank}) \div (A_{Standard} - A_{Blank}) \times C_{Standard} \div (W \div V) \times f$$

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 Data

▲Example analysis

For 10% mango tissue homogenate, dilute for 200 times, then take 200 uL sample and carry the assay according to the operation table. 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