Sucrose Assay Kit

Cat#:orb1499608 (manual)

Microassay

Size: 100T/96S

Product composition and storage conditions :

No.	Specifications	Storage Conditions
ES428	100 ml ×1 bottle	4 °C
orb1499608- A	Powder ×1 bottle	4 °C, add 1 mL distilled water to dissolve before use, then diluten 10 times with distilled water, and the final concentration is 1 mg/mL
orb1499608- B	2mL×1 bottle	4 °C
orb1499608- C	20mL×1 bottle	4 °C
orb1499608- D	5ml ×1 bottle	4 °C away from light
orb1499608- E	Powder 0.5g ×1 bottle	RT

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction :

Significance: Sucrose is the main product of photosynthesis and the main form of sugar transport and storage. Therefore, the determination of sucrose content is of great significance for plant sugar metabolism. In addition, sucrose content is one of the important indexes for quality control of beverage, honey, preserved fruit, candy and dairy products.

Principle: The reducing sugar is destroyed by co-heating the sample with alkali. Sucrose is then hydrolyzed into glucose and fructose under acidic conditions, and fructose further reacts with resorcin to form colored substances with characteristic absorption peaks at 480 nm.

Own supplies :

Visible spectrophotometer or microplate reader, water bath, adjustable pipette, micro quartz cuvette/96-wells plate and distilled water.

Sample processing :

1. Weigh 0.1~0.2 g sample, grind at room temperature, add 0.5mL ES428, transfer to centrifuge tube quickly after proper grinding, place in 80℃ water bath for 10 min, shake for 3~5 times, centrifuge with 4000 g at room temperature for 10 min

after cooling, take supernatant, add 2 mg orb1499608-E, decolorize at 80℃ for 30 min, add 0.5mL ES428, centrifuge with 4000g at room temperature for 10 min after coolilng, take supernatant for determination.

Measurement steps:

1. Preheat the visible spectrophotometer or microplate reader for at least 30 minutes, adjust the wavelength to 480nm, set zero with distilled water.
2. Sample determination: add the following reagents in sequence to the EP tube :

Reagent name	Blank tube (μl)	Standard tube (μl)	Measuring tube (μl)
Sample			25
orb1499608- A		25	
Distilled water	25		
orb1499608- B	15	15	15
Mix well, boil in boiling water bath for about 5 min(Cover tightly to prevent water loss)			
orb1499608- C	175	175	175
orb1499608- D	50	50	50
Mix well, boil in a boiling water bath for 30 min, take 200 μL after cooling to a microquartz cuvette or 96well plate, measure the light absorption value at 480nm, and record the blank tube, standard tube and measuring tube as A1, A2 and A3 respectively. Note: the blank tube and the standard tube are only need to test one tube.			

Sucrose content calculation:

1. Calculated by protein concentration
Sucrose content (mg/mg prot) = (C standard×V1)×(A3-A1)÷(A2-A1) ÷(V1×Cpr) = (A3-A1)÷(A2-A1)÷Cpr

2. Calculated according to sample quality
Sucrose content (mg/g fresh weight) = (C standard×V1)×(A3-A1)÷(A2-A1)÷(W×V1÷V2) = (A3-A1)÷(A2-A1) ÷W

Note: C standard: Standard tube concentration, 1 mg/mL; V1: Sample volume added, 0.025mL; V2: Extraction solution (ES428) volume added, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Fresh weight of sample, g.

Precautions:
When the absorbance of the sample is greater than 1.0, it is recommended that the sample be diluted with the extract solution and then determined. Pay attention to multiply by the dilution times in the formula.