Plant Tissue Fructose Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Microplate Reader/Spectrophotometer

Catalog Number: BC2455

Size:100T/48S

Components:

Name of reagent	Size	storage
Extract Solution	Liquid 60 mL × 1 bottle	2-8 ℃
Reagent **I**	Liquid 1.25 mL × 1 bottle	2-8 ℃
Reagent II	Liquid 21 mL × 1 bottle	2-8 ℃
Reagent III	Liquid 7 mL × 1 bottle	2-8 ℃
Reagent IV	Powder × 1 bottle	RT
Standard	Powder × 1 bottle	2-8 ℃

Preparation of solution:

1. Standard: Add 0.695mL distilled water to dissolve before use to obtain 80 mmol/L fructose standard, and store it at 2-8 ℃ for 4 weeks;
2. Preparation of 8mmol/L standard: take 100μL 80mmol/L standard, add 900μL distilled water, fully mix. prepare 8mmol/L standard for standard tube determination

Product Description:

Fructose is one kind of ketohexose and an isomeride of glucose, which exists widely in the pμlp of fruits and honey with free state. It can be combined with glucose to form sucrose. Fructose is the sweetest monosaccharide and is used widely in the production of food, medicine and health care products.

In acidic conditions, fructose reacts with resorcinol to form colored substances which has characteristic absorption peaks at 480 nm.

Reagents and Equipments Required but Not Provided:

Spectrophotometer/ microplate reader, water bath, centrifuge, adjustable transferpettor, micro glass cuvette/ 96 well flat-bottom plate, mortar/homogenizer, distilled water.

Operation procedure:

I. Sample Preparation

（1） Weigh about 0.05g sample and grind it at room temperature; Add 0.5mL of extract Solution, grind it properly and transfer it to the covered centrifuge tube quickly;

（2） Place it in a water bath at 80 ℃ for 10min (wrap sealing film to prevent lid explosion), shake it for 3-5 times, cool it to room temperature, centrifuge it at 4000g at room temperature for 10min, and take 0.4mL of supernatant;

（3） Add a small amount (about 2mg) of reagent 4 and decolor at 80 ℃ for 30min (wrap sealing film to prevent cap explosion);

（4） Then add 0.4mL of extract Solution, 4000g, centrifuge at RT for 10min, and take the supernatant for determination.

Note: ① If there is any volume change, the supernatant in step (2) and the extract in step (4) shall be added according to the volume ratio of 1:1;

② To prevent explosion cover, you can use the spiral mouth EP tube or with a lock buckle EP tube （wrapped sealing film）, you can also tied 1 hole on the cover of ordinary EP tube (wrapped sealing film)

II. Detection

1. Preheat spectrophotometer/ microplate reader for 30 min, adjust the wavelength to 480 nm, and set spectrophotometer counter to zero with distilled water.

2. Add these reagents to a 1.5 mL EP tube:

Reagent name (μL)	Test tube (A_T)	Control tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Sample	30	30	-	-
Standard	-	-	30	-
Distilled water	20	-	20	50
Reagent I	-	20	-	-
-	-	Water bath at 100 ℃ for 10min	-	-
Reagent II	190	190	190	190
Reagent III	60	60	60	60
Vortex and mix evenly, place in a 100 ℃ water bath for accurate reaction for 10min (wrap sealing film to prevent cover explosion), cool to room temperature, take 200 μL to micro glass cuvette/96 well flat-bottom plate and detect absorbance at 480 nm after cooling, record as A_T, A_C, A_S, A_B, and calculate $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$. Standard tube and blank tube only need to measure 1-2 times.				

Calculation:

1. Protein concentration:

Fructose (mg/mg prot) = $C \times \Delta A \div \Delta As \times Ve \div (Cpr \times Ve) \times 180 \times F = 1440 \times \Delta A \div \Delta As \div Cpr \times F$

2. Sample weight:

Fructose (mg/g fresh weight) = $C \times \Delta At \div \Delta As \times Ve \div W \times F = 1.44 \div \Delta A \div \Delta As \div W \times F$

C: Standard concentration, 8mmol/L;

Cpr: Sample concentration (mg/mL);

W: Sample weight(g);

Ve: Extraction volume, 1×10^{-3} L

180: Fructose Relative molecular mass, 180mg/mmol

Note:

1. If the ΔA is greater than 1.1, it is recommended that the sample be diluted with water for determination. Pay attention to multiplying the dilution multiple in the calculation formula; if the ΔA is less than 0.02, it is recommended to increase the sample mass and re-extract.

2. Boiling or high temperature process can use spiral mouth EP tube or with lock buckle EP tube and wrap sealing film; also can be wrapped in the film of ordinary EP tube cover 1 holes to prevent explosion.

3. The protein has been removed during the pretreatment process. If the protein concentration is used to calculate, another tissue shall be taken for re-extraction.

Experimental examples:

1. Take 0.1039g of mango fruit, carry out pretreatment according to the sample treatment steps, take the supernatant and dilute it 4 times according to the determination steps, measure and calculate ΔA = 0.549-0.093 = 0.456, ΔA_S=0.434-0.05=0.384, and calculate according to the sample mass:
   Fructose content (mg/g mass) = 1.44 × ΔA÷ ΔA_S÷ W×F = 65.833 mg/g mass.
2. Take 0.1032g carrot, carry out pretreatment according to the sample treatment steps, take supernatant, dilute 4 times according to the determination steps, measure with 1mL glass cuvette and calculate ΔA = 0.47- 0.127 = 0.343, ΔA_S = 0.434-0.05=0.384, and calculate according to the sample mass:
   Fructose content (mg/g mass) = 1.44 × ΔA÷ ΔA_S÷ W×F = 48.722mg/g mass.

References:

[1] Varandas S, Teixeira M J, Marques J C, et al. Glucose and fructose levels on grape skin: interference in Lobesia botrana behaviour[J]. Analytica chimica acta, 2004, 513(1): 351-355.

Related Products:

BC0340/BC0345 Glycogen Assay Kit
BC2460/BC2465 Plant Sucrose Content Detection Kit
BC2500/BC2505 Glucose Assay Kit
BC0330/BC0335 Trehalose Content Assay Kit