Sucrose Assay Kit

Catalog No.: abx298802

Size: 96 tests

Detection Range: 0.32 µmol/ml – 70 µmol/ml

Sensitivity: 0.32 µmol/ml

Storage: Store all components at 4°C.

Application: For detection and quantification of Sucrose content in plant tissue.

Introduction

Abbexa's Sucrose Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Sucrose content. Sucrose in plant tissue is hydrolyzed into glucose and fructose at high temperature under acidic conditions. Fructose is converted by dehydration to 5-hydroxymethylfurfural. Glucose must be first isomerized into Fructose and then into 5-hydroxymethylfurfural. Sample Sucrose content is calculated using absorbance of 5-hydroxymethyl furfural at 290m, this value is mostly attributed to Fructose content, as the rate of hydrolysis of Glucose to Fructose is slow.

Kit components

1. Detection Reagent solution: 4 × 60 ml
2. Sucrose standard (100 µmol/ml): 1 ml

Materials required but not provided

1. Spectrophotometer (290 nm) and cuvettes (1 cm optical path)
2. Double-distilled water
3. Saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vortex mixer
7. Water bath (100 °C)

Protocol

A. Preparation of samples and reagents

1. Samples

- **Tissue Homogenates:** Wash 0.02-1 g of tissue with pre-chilled PBS (0.01M pH 7.4) and dry with absorbent paper. For each 1 g of tissue, add 9 ml of pre-chilled PBS (0.01M pH 7.4). Mince tissues to small pieces, homogenize by hand, using a mechanical homogenizer, or by ultrasonication (60 Hz, 90s), on ice. Homogenization time may be increased according to sample type as necessary. Centrifuge the homogenate at 3100 x g at 4°C for 10 min. Collect the supernatant on ice and assay immediately. If a precipitate appears, centrifuge again. The protein concentration in the supernatant should be determined separately **(abx097193)**. Non-homogenized tissue samples may be stored safely at -80 ºC for up to one month.

  It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Green pepper tissue homogenate	1
10% *Epiprimnum aureum* tissue homogenate	1
10% Cucumber tissue homogenate	1

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical homogenization methods.
- Where required, samples should be diluted using PBS (0.01M pH 7.4).

2. Reagents

- **Sucrose standard (20 µmol/ml):** Dilute Sucrose standard (100 µmol/ml) with double-distilled water to a 1:4 ratio (for example, add 1 ml of 100 µmol/ml Sucrose standard to 4 ml of double-distilled water and mix fully). Prepared standard solution may be stored at 4°C for up to one week.

B. Assay Procedure

1. Set blank, standard and sample tubes, and label accordingly.
2. Add 0.03 ml double-distilled water to each blank tube.
3. Add 0.03 ml of 20 µmol/ml Sucrose standard to each standard tube.
4. Add 0.03 ml of sample to each sample tube.
5. Add 2 ml of Detection Reagent solution to all tubes and mix fully with a vortex mixer.
6. Seal the tube with film and pierce a small hole in the film.
7. Incubate tubes in a water bath at 100°C for 8 min accurately.
8. Cool tubes under running water.
9. Zero the spectrophotometer using double-distilled water, then measure OD values at 290 nm using 1 cm optical path quartz cuvettes.

Notes:

- Water bath temperature must remain constant > 95°C
- Only glass tubes may be used for the reaction
- Absorbance values must be detected within 20 min.

C. Calculation of Results

$$\text{Sucrose (µmol/mg)} = \frac{\Delta \mathbf{A_1} \times \boldsymbol{C_{st}}}{\Delta \mathbf{A_2} \times \boldsymbol{C_{pr}}} \times \boldsymbol{f}$$

where:

$\Delta \mathbf{A_1}$	$\mathbf{OD_{Sample} - OD_{Blank}}$
$\Delta \mathbf{A_2}$	$\mathbf{OD_{Standard} - OD_{Blank}}$
$\boldsymbol{f}$	Sample dilution factor
$\boldsymbol{C_{pr}}$	Sample protein concentration (mg protein)
$\boldsymbol{C_{st}}$	Concentration of the standard (20 µmol/ml)