Sucrose Synthase Assay Kit

Catalog No.: abx298804

Size: 96 tests

Detection Range: 100 µg/ml - 4000 µg/ml

Storage: Store all kit components at 4°C.

Application: For detection and quantification of Sucrose Synthase activity in tissue homogenates and other biological fluids.

Introduction

Abbexa's Sucrose Synthase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Sucrose Synthase activity. The reaction product has an absorbance maxima at 480 nm. The intensity of the color is proportional to the Sucrose Synthase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer: 4 × 30 ml
3. Substrate: 1 vial
4. Substrate Diluent: 3 ml
5. Reaction Buffer: 10 ml
6. Stop Solution: 1 ml
7. Dye Reagent: 1 vial
8. Standard: 4 mg
9. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (480 nm)
2. Distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Mortar or homogenizer
6. Centrifuge and centrifuge tubes
7. Vortex mixer
8. Incubator (30 °C)
9. Heated water bath
10. Ice

Protocol

A. Preparation of Samples and Reagents

1. Reagents

- **Dye Reagent Solution:** Add 5 ml of distilled water into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.
- **Standard Solution:** Add 1 ml of distilled water into the Standard vial and mix thoroughly to prepare the Standard Solution (concentration 4 mg/ml = 4000 µg/ml). Ensure that the Standard has completely dissolved prior to use.
- **Substrate Solution:** Add 3 ml of Substrate Diluent into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

2. Samples

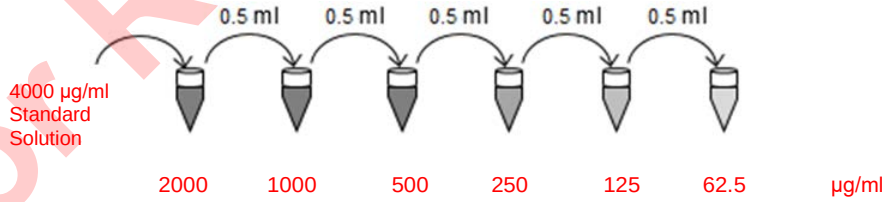
- **Tissue Samples:** Homogenize 0.1 g of sample on ice in 1 ml of Assay Buffer. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.
- **Liquid Samples:** Liquid samples can be used directly or diluted in Assay Buffer.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentrations within the detection range of the kit.

1. Label 6 tubes with 2000 µg/ml, 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 4000 µg/ml Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set sample, standard and blank microcentrifuge tubes.
3. Add 10 µl of sample to each of the sample tubes.
4. Add 10 µl of prepared standard solutions to the standard tubes.
5. Add 10 µl of distilled water to the blank tubes.
6. Add 30 µl of Substrate Solution to all tubes. Mix thoroughly.
7. Incubate all tubes at 30°C for 30 minutes.
8. Add 10 µl of Stop Solution to all tubes. Mix thoroughly.
9. Place all tubes in a boiling water bath for 10 minutes, then place on ice.
10. Add 100 µl of Reaction Buffer to all tubes.
11. Add 50 µl of Dye Reagent Solution to all tubes. Mix thoroughly.

12. Place all tubes in a boiling water bath for 5 minutes, then centrifuge the tubes.
13. Set sample, standard and blank wells on the microplate and record their positions. Transfer the supernatant of each tube into the corresponding well on the microplate.
14. Measure the OD of each well with a microplate reader at 480 nm.

C. Calculation of Results

One unit of Sucrose Synthase activity is defined as the quantity of enzyme required to generate 1 μg of sucrose per minute.

1. Activity per protein concentration of sample

$$\text{Sucrose Synthase } (\text{U/mg}) = \frac{C_{Standard} \times V_{Standard}}{C_{Protein} \times V_{Sample} \times T} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{400}{C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

2. Activity per gram of sample

$$\text{Sucrose Synthase } (\text{U/g}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{W \times V_{Sample} \times T} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{400}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

OD_{Blank}	OD value of blank
$OD_{Standard}$	OD value of the highest standard
OD_{Sample}	OD value of sample
$C_{Protein}$	Concentration of protein in sample (mg/ml)
$C_{Standard}$	Concentration of the highest standard (4 mg/ml = 4000 μg/ml)
W	Weight of sample (g)
T	Reaction time (10 minutes)
$V_{Standard}$	Volume of the standard (0.01 ml)
V_{Sample}	Volume of the sample (0.01 ml)
V_{Assay}	Volume of the Assay Buffer (1 ml)