Sucrase Assay Kit

Catalog No.: abx298803

Size: 96 tests

Detection Range: 20 U/ml – 2000 U/ml

Sensitivity: 20 U/ml

Storage: Store all components at 4°C. Store the Phenol Solution and Enzyme Solution in the dark.

Application: For detection and quantification of Sucrase activity in tissue homogenates.

Introduction

Abbexa's Sucrase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Sucrase activity. Sucrase converts sucrose into glucose. The enzyme glucose oxidase is added, which breaks down glucose to release hydrogen peroxide. Hydrogen peroxide reacts with a chromogenic compound to produce a red substance, with an absorbance maximum at 505 nm. The intensity of the color is proportional to the Sucrase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer: 10 ml
3. Phenol Solution: 12 ml
4. Enzyme Solution: 12 ml
5. Stop Reagent: 1 vial
6. Standard (50 mmol/L): 1 ml
7. Substrate: 1 vial
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (505 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the tissue into PBS (0.01 M, pH 7.4) in a ratio of 1 : 4 mass (mg) to volume (µl) (e.g. for 20 mg of tissue, add into 80 µl of cold PBS). Homogenize manually, using a mechanical homogenizer, at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice, and assay immediately.

Note: To calculate Sucrase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

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 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
20% Rat ileum tissue homogenate	1
20% Rat stomach tissue homogenate	1
20% Rat liver tissue homogenate	1

Note:

- 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 lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate Working Solution:** Reconstitute 1 vial of Substrate with 8 ml of Assay Buffer. Mix fully until dissolved. The prepared Substrate Working Solution can be stored for up to 7 days at 4°C.
- **Working Stop Solution:** Reconstitute 1 vial of Stop Reagent with 5 ml double-distilled water. Mix fully until dissolved. The prepared Working Stop Solution can be stored for up to 7 days at 4°C.

- **Chromogenic Solution:** Prepare only as much solution as required by the number of wells tested.

  For each well, prepare 200 µl Chromogenic Solution by mixing 100 µl of Phenol Solution and 100 µl of Enzyme Solution. Mix fully. The Chromogenic Solution should be prepared just before use, and should not be stored.

- **Standards:** Label 7 tubes with 25 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L, 2 mmol/L, and 1 mmol/L. Add 250 µl, 200 µl, 150 µl, 100 µl, 50 µl, 20 µl, and 10 µl of Standard (50 mmol/L) to the 25 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L, 2 mmol/L, and 1 mmol/L tubes respectively, followed by 250 µl, 300 µl, 350 µl, 400 µl, 450 µl, 480 µl, and 490 µl of double-distilled water, to prepare Standard Dilutions with concentrations 25 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L, 2 mmol/L, and 1 mmol/L. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	**25**	**20**	**15**	**10**	**5**	**2**	**1**
50 mmol/L Standard (µl)	250	200	150	100	50	20	10
Double-distilled water (µl)	250	300	350	400	450	480	490

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 500 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- When preparing the Chromogenic Solution, take extra care not to contaminate the Enzyme Solution stock vial.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark microcentrifuge tubes for each standard, sample, and control. Each sample requires a corresponding control tube. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 25 µl of the standard dilutions into the corresponding standard tubes.
3. Add 25 µl of each sample into the sample tubes.
4. Add 50 µl of Substrate Working Solution into the standard, sample, and control tubes.
5. Mix fully, then incubate all tubes at 37°C for 20 minutes. *This step must be timed precisely.*
6. Add 25 µl of Working Stop Solution to all tubes. Mix fully.
7. Add 25 µl of sample to the corresponding control tubes.
8. Mix fully, then centrifuge at 1780 × g for 10 minutes. Set positions on the 96-well plate for each standard, sample, and control.
9. Take 8 µl of supernatant from each tube, and add to the corresponding well on the 96-well microplate.
10. Add 200 µl of Chromogenic Solution to all wells.
11. Tap or shake the plate gently for 10 seconds to mix fully. Incubate at 37°C for 15 minutes.
12. Measure the OD of each well with a microplate reader at 505 nm.

C. Calculation of Results

Plot the standard curve, using the mean OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Sucrase in each sample well can be derived with the following formula:

1. Tissue samples:

One unit of Sucrase activity is defined as the amount required for 1 mg of tissue protein to consume 1 nmol of sucrose per minute at 37°C.

$$\text{Sucrase (U/mg protein)} = \mathrm{F} \times 1000 \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Control}} - \mathrm{b})}{\mathrm{a} \times \mathrm{t} \times \mathrm{C_{Protein}}}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Control}}$	OD value of the sample's corresponding control
$\mathrm{C_{Protein}}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
t	Time of the enzymatic reaction (20 mins)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.