Technical Manual

Sucrase Activity Assay Kit

- Catalogue Code: MAES0192
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

20-2000 U/mL

Sensitivity:

20 U/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Sucrase is one of the glycosidases, which can specifically catalyze the hydrolysis of sucrose to glucose and fructose. It is widely found in animal, plant and microorganisms and plays a key role in carbohydrate metabolism of animals and plants.

3. Intended Use

This kit can be used to measure sucrase activity in animal tissue samples.

4. Detection Principle

Sucrase catalyzes its substrate (sucrose) to produce glucose, which produces hydrogen peroxide under the action of glucose oxidase. Hydrogen peroxide reacts with chromogenic agent to produce red substance, which has a strong absorption peak at 505 nm. In a certain concentration range, Its absorbance is proportional to glucose concentration. Therefore, the activity of sucrase can be calculated by measuring the OD value at 505 nm.

5. Kit Components & Storage

Item	Specification	Storage
Substrate	Lyophilized × 1 vial	2-8°C, 6 months
Buffer Solution	10 mL × 1 vial	2-8°C, 6 months
Phenol Solution	12 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Enzyme Solution	12 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Stop Solution	Lyophilized × 1 vial	2-8°C, 6 months
Glucose Standard (50 mmol/L)	1 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Centrifuge
- Microplate Reader (500-520 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. Control the time of enzymatic reaction strictly.

2. Prevent contaminating enzyme solution when preparing of chromogenic solution.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution**: Dissolve substrate with 8 mL of buffer solution and shake until substrate is dissolved fully. The prepared solution can be stored at 2-8°C for 7 days.

3. Preparation of **chromogenic solution**: Mix the phenol solution and enzyme solution at a ratio of 1:1. Prepare the fresh solution before use.

4. Preparation of **stop working solution**: Dissolve stop solution with 5 mL of ultrapure water and shake until stop solution is dissolved fully. The prepared solution can be stored at 2-8°C for 7 days.

8. Sample Preparation

Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (20-2000 U/mL).

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: The diluent is PBS (0.01 M, pH 7.4).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 505 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

10. Operation Steps

The preparation of standard curve

Dilute 50 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 5, 10, 15, 20, 25 mmol/L.

The measurement of samples

1. **Standard tube:** Take 25 μL of standards with different concentrations into 1.5 mL EP tubes.
   Sample tube: Take 25 μL of sample into 1.5 mL EP tubes.
   Control tube: Take 50 μL of substrate working solution into 1.5 mL EP tubes.

2. Add 50 μL of substrate working solution into the standard tubes and sample tubes.

3. Mix fully and incubate at 37°C for 20 min.

4. Add 25 μL of stop solution into each tube and mix fully.

5. Add 25 μL of sample into control tubes.

6. Mix fully, centrifuge at 1780 g for 10 min and take 8 μL of supernatant from each tube to the corresponding wells.

7. Add 200 μL of chromogenic solution into each well.

8. Mix fully with microplate reader for 10 s, incubate at 37°C for 15 min. Measure the OD values of each well at 505 nm with microplate reader.

Operation Table

	Standard tube	Sample tube	Control tube
Standards with different concentrations (μL)	25		
Sample (μL)		25	
Substrate working solution (μL)	50	50	50
Mix fully and incubate at 37°C for 20 min.			
Stop solution (μL)	25	25	25
Sample (μL)			25
Mix fully, centrifuge at 1780 g for 10 min and take 8 μL of supernatant from each tube to the corresponding wells.			
Supernatant (μL)	8	8	8
Chromogenic solution (μL)	200	200	200
Mix fully with microplate reader for 10 s, incubate at 37°C for 15 min. Measure the OD values of each well at 505 nm with microplate reader.			

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

Definition: The amount of 1 nmol sucrose hydrolysised by 1 g tissue protein per minute at 37°C is defined as 1 activity unit.

$$\text{Sucrase activity (U/mgprot)} = \frac{\Delta A - b}{a} \div T \times 1000^{*} \times f \div C_{pr}$$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).
x: The concentration of standard.
a: The slope of standard curve.
b: The intercept of standard curve.
ΔA: $OD_{Sample} - OD_{Control}$.
T: Enzymatic reaction reaction time, 20 min.
1000*: 1 μmol = 1000 nmol.
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, mgprot/mL

12. Performance Characteristics

Detection Range	20-2000 U/mL
Sensitivity	20 U/mL
Average recovery rate (%)	100
Average inter-assay CV (%)	6.5
Average intra-assay CV (%)	5.4

Analysis

For rat ileum tissue, take 25 μL of rat ileum tissue supernatant and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0306 x + 0.0025, the average OD value of the sample is 0.204, the average OD value of the control is 0.079, the concentration of protein in sample is 6.48 mgprot/mL, and the calculation result is:

$$\text{Sucrase activity (U/mgprot)} = (0.204 - 0.079 - 0.0025) \div 0.0306 \div 20 \times 1000 \div 6.48$$

$$= 30.89 \text{ U/mgprot}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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