Sucrase Activity Assay Kit

Catalog No: E-BC-K751-M

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 20 U/mL

Detection range: 20-2000 U/mL

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Substrate	Powder × 1 vial	2-8°C , 6 months
Reagent 2	Buffer Solution	10 mL × 1 vial	2-8°C , 6 months
Reagent 3	Phenol Solution	12 mL × 1 vial	2-8°C , 6 months, shading light
Reagent 4	Enzyme Solution	12 mL × 1 vial	2-8°C , 6 months, shading light
Reagent 5	Stop Solution	Powder × 1 vial	2-8°C , 6 months
Reagent 6	50 mmol/L Glucose Standard	1 mL × 1 vial	2-8°C , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Micropipette, Vortex mixer, Centrifuge, Microplate reader (500-520 nm, optimum wavelength: 505 nm)

Reagents

Double distilled water, PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key point of the assay

1. Control the time of enzymatic reaction strictly.
2. Avoid contaminating reagent 4 when preparing of chromogenic solution.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of reagent 1 working solution:
   Dissolve reagent 1 with 8 mL of reagent 2 and shake until reagent 1 is dissolved fully. The prepared solution can be stored at 2-8°C for 7 days.
3. Preparation of chromogenic solution:
   Mix the reagent 3 and reagent 4 at a ratio of 1:1. Prepare the fresh solution before use.
4. The preparation of reagent 5 working solution:
   Dissolve reagent 5 with 5 mL of ultrapure water and shake until reagent 5 is dissolved fully. The prepared solution can be stored at 2-8°C for 7 days.

▲Sample preparation

Tissue homogenate: Accurately weigh 0.05-1 g fresh tissue sample, add 4 times the volume of PBS (0.01 M, pH7~7.4) according to the ratio of Weight (g): Volume (mL) =1:4. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g at 4°C for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, stored the serum at-80°C , which can be stored for a month. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	505 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don’t add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲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.

▲Operating steps

1.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.

2.The measurement of sample

1) Standard tube: Take 25 μL of standards with different concentrations into 1.5 mL EP tubes.

Sample tube: Take 25 μL of sample into1.5 mL EP tubes.

Control tube: Take 50 μL of reagent 1 working solution into 1.5 mL EP tubes.

2) Add 50 μL of reagent 1 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 reagent 5 into each tube and mix fully.
Add 25 μL of sample into control tubes.
5) Mix fully, centrifuge at 1780 g for 10 min and take 8 μL of supernatant from each tube to the corresponding wells.
6) Add 200 μL of chromogenic solution into each well.
7) 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 well	Sample well	Control well
Standards with different concentrations (μL)	25		
Sample (μL)		25	
Reagent 1 working solution (μL)	50	50	50
Mix fully and incubate at 37°C for 20 min.			
Reagent 5 (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.			

▲Calculation

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.

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

Sucrase activity (U/mgprot) = (ΔA - b) ÷ a ÷T ×1000* × f ÷ C_{pr}

Note:

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 tissue sample, mgprot/mL.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Example 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:

Sucrase activity (U/mgprot) = (0.204-0.079-0.0025) ÷0.0306 ÷20×1000÷6.48
=30.89 U/mgprot