（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K751-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（500-520 nm）

Detection range：20-2000 U/mL

Elabscience® Sucrase Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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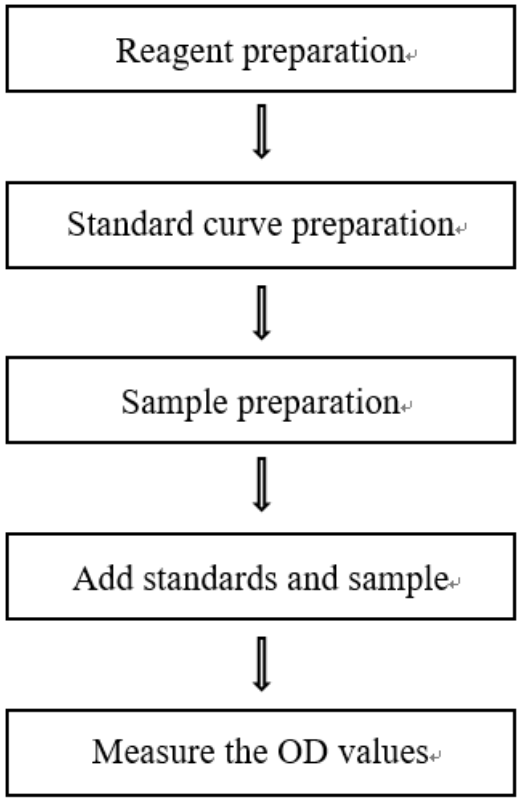
Assay summary



Intended use

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

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, It's 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	Size (96 T)	Storage
Reagent 1	Substrate	Powder ×1 vial	2-8℃, 12 months
Reagent 2	Buffer Solution	10 mL ×1 vial	2-8℃, 12 months
Reagent 3	Phenol Solution	12 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 4	Enzyme Solution	12 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 5	Stop Solution	Powder ×1 vial	2-8℃, 12 months
Reagent 6	50 mmol/L Glucose Standard	1 mL ×1 vial	2-8℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution:

Dissolve one vial of substrate with 8 mL of buffer solution, mix well to dissolve. Store at 2-8℃ for 7 days.

③ Preparation of stop working solution:

Dissolve one vial of stop solution with 5 mL of ultrapure water, mix well to dissolve. Store at 2-8℃ for 7 days.

④ Preparation of chromogenic solution:

For each well, prepare 200 μL of chromogenic solution (mix well 100 μL of phenol solution and 100 μL of enzyme solution). The chromogenic solution should be prepared on spot.

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**1**	**2**	**5**	**10**	**15**	**20**	**25**
50 mmol/L standard (μL)	0	10	20	50	100	150	200	250
Double distilled water (μL)	500	490	480	450	400	350	300	250

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 80 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Control the time of enzymatic reaction strictly.

② Avoid contaminating enzyme solution when preparing of chromogenic solution.

Operating steps

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 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℃ for 20 min.

④ Add 25 μL of stop working solution 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℃ for 15 min. Measure the OD values of each well at 505 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue sample:

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

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

[Note]

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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	32.50	135.00	482.00
%CV	5.7	5.3	5.2

Inter-assay Precision

Three rat liver tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	32.50	135.00	482.00
%CV	6.2	6.4	6.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	1.5	7.8	17.5
Observed Conc. (mmol/L)	1.5	8.0	16.8
Recovery rate (%)	101	103	96

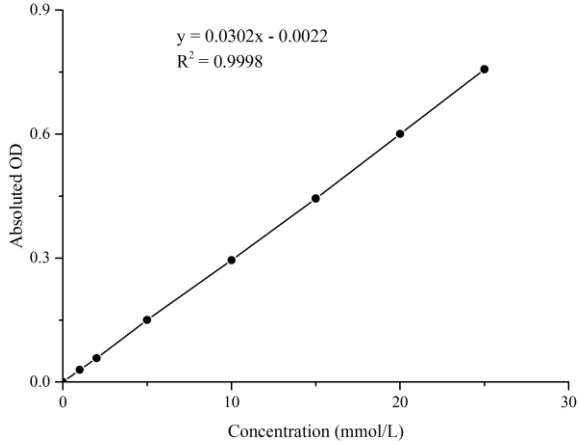
Sensitivity

The analytical sensitivity of the assay is 20 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**1**	**2**	**5**	**10**	**15**	**20**	**25**
Average OD	0.083	0.112	0.140	0.233	0.378	0.527	0.683	0.840
Absoluted OD	0.000	0.030	0.058	0.150	0.295	0.444	0.600	0.757



Appendix II Example Analysis

Example analysis：

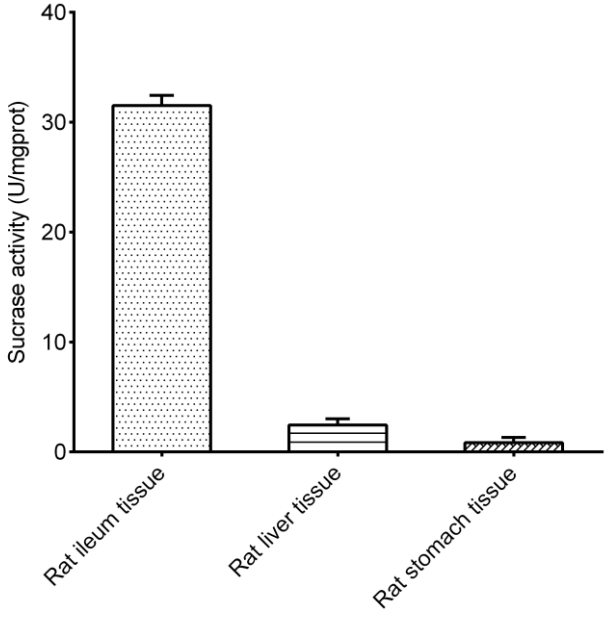
For rat ileum tissue, take 25 μL of rat ileum tissue supernatant and carry the assay according to the operation table. The results are as follows:

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:

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

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

Detect 20% rat ileum tissue homogenate (the concentration of protein is 6.48 mgprot/mL), 20% rat liver tissue homogenate (the concentration of protein is 23.65 mgprot/mL) and 20% rat stomach tissue homogenate (the concentration of protein is 5.89 mgprot/mL) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.