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

Catalog No：E-BC-K131-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（495-510 nm）

Detection range：12.5-2000 U/mL

Elabscience® Lactase 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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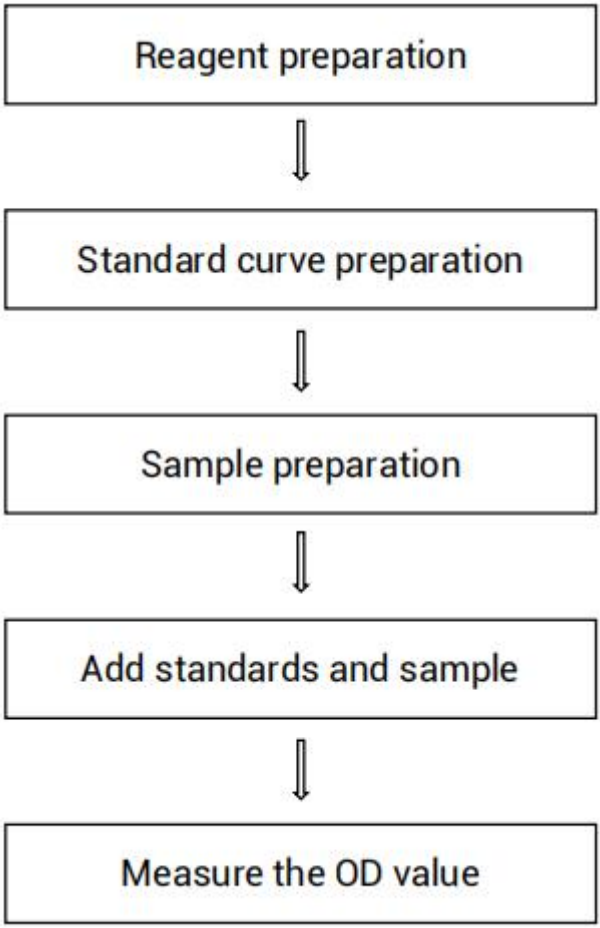
Assay summary



Intended use

This kit can measure lactase activity in animal tissue samples.

Detection principle

Lactase decomposes lactose to produce glucose. Under the action of enzyme, glucose produces hydrogen peroxide. In the presence of chromogenic oxygen receptors, peroxidase catalyzes hydrogen peroxide to produce colored substances. Lactase activity 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°C, 12 months
Reagent 2	Buffer Solution	10 mL × 1 vial	2-8°C, 12 months
Reagent 3	Stop Solution	6 mL × 1 vial	2-8°C, 12 months
Reagent 4	Phenol Solution	12 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 5	Enzyme Solution	12 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 6	50 mmol/L Glucose Standard Solution	1.5 mL × 1 vial	2-8°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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:

Micropipettor, Vortex mixer, Centrifuge, Water bath, Incubator, Microplate reader (495-510 nm, optimum wavelength 505 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution:
Dissolve one vial of substrate with 8 mL of buffer solution, mix well. Store at 2-8°C for 1 month protected from light.

③ The preparation of chromogenic agent:
For each well, prepare 200 μL of chromogenic agent (100 μL of phenol solution and 100 μL of enzyme solution). The chromogenic agent should be prepared on spot.

④ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 50 mmol/L glucose standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 5, 10, 15, 20, 30, 40 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**2**	**5**	**10**	**15**	**20**	**30**	**40**
50 mmol/L standard (μL)	0	4	10	20	30	40	60	80
Double distilled water (μL)	100	96	90	80	70	60	40	20

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 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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
10% Rat lleal tissue homogenate	1
10% Rat jejunum tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The temperature and time of incubation at 37°C must be accurately.

② If the lactase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

③ Accurate operation is required when adding liquid to microplate and prevent the formulation of bubbles when adding the liquid to the microplate.

Operating steps

① Standard tube: add 25 μL of standard solution with different concentrations to the corresponding 1.5 mL EP tubes.

Sample tube: add 25 μL of sample to the corresponding 1.5 mL EP tubes.

Control tube: add nothing.

② Add 50 μL of substrate working solution to each tube.

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

④ Add 25 μL of stop solution to each tube.

⑤ Standard tube: add nothing.

Sample tube: add nothing.

Control tube: add 25 μL of sample to the corresponding 1.5 mL EP tubes.

⑥ Mix fully and centrifuge at 1780×g for 10 min.

⑦ Take 8 μL of the supernatant to corresponding wells in microplate.

⑧ Add 200 μL of chromogenic agent to each well.

⑨ Mix fully for 10 s with microplate reader, incubate at 37°C for 15 min and measure the OD value of each well at 505 nm.

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:

Definition: The amount of 1 mmol of lactose hydrolyzed by 1 mg of tissue protein per minute at 37 °Cis defined as 1 unit.

$$\text{Lactase activity (U/mgprot)} = (\Delta A - b) \div a \div 20^{*} \times 1000^{**} \times f \div C_{pr}$$

[Note]

f: Dilution factor of sample before tested.

ΔA: $OD_{Sample} - OD_{Control}$.

20*: Reaction time, 20 min

1000**: 1 μmol=1000 nmol.

C_{pr}: The concentration of protein in sample, mgprot/mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	25.00	103.00	254.00
%CV	4.8	4.5	4.2

Inter-assay Precision

Three human serum 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)	25.00	103.00	254.00
%CV	8.2	8.6	8.7

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 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	4.5	12	26
Observed Conc. (mmol/L)	4.5	11.9	27.6
Recovery rate (%)	101	99	106

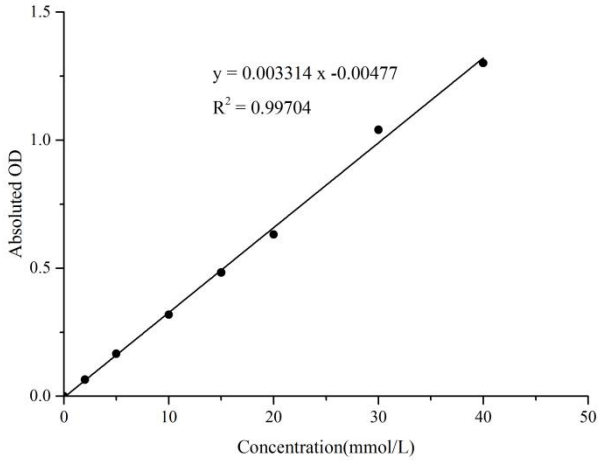
Sensitivity

The analytical sensitivity of the assay is 3.94 U/L. 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	2	5	10	15	20	30	40
OD value	0.049	0.115	0.218	0.368	0.531	0.687	1.140	1.323
	0.048	0.112	0.210	0.366	0.532	0.674	1.037	1.375
Average OD	0.048	0.113	0.214	0.367	0.531	0.680	1.089	1.349
Absoluted OD	0.000	0.065	0.166	0.319	0.483	0.632	1.040	1.301



Appendix Π Example Analysis

Example analysis：

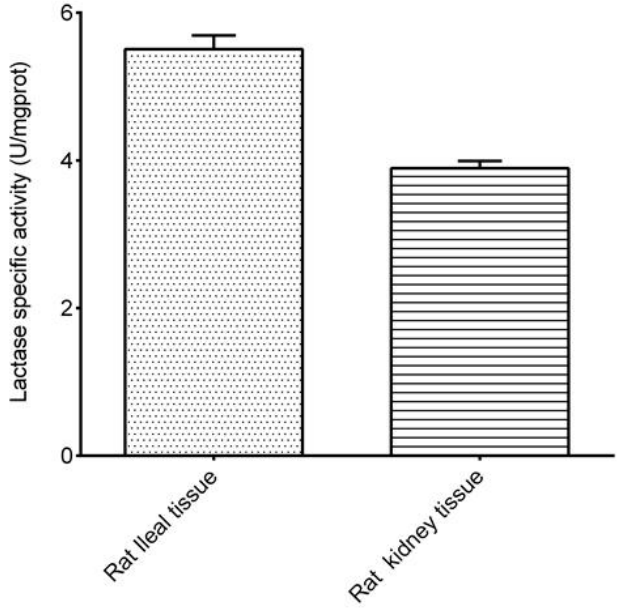
For rat lleal tissue, take 25 μL of 10% rat lleal tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: y =0.0342 x - 0.0078, the average OD value of the sample is 0.065, the average OD value of the blank is 0.053, the concentration of protein in sample is 5.19 mgprot/mL, and the calculation result is:

Lactase activity (U/mgprot) = (0.065 – 0.053 + 0.0078) ÷ 0.0342 ÷ 20 × 1000 ÷ 5.19

= 5.58 U/mgprot

Detect 10% rat lleal tissue homogenate (the concentration of protein is 5.19 mgprot/mL L) and 10% rat kidney tissue homogenate (the concentration of protein is 9.50 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.