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

Catalog No：E-BC-K865-S

Specification：100 Assays (42 samples)

Measuring instrument：Spectrophotometer (495-510 nm)

Detection range：0.10-125 U/L

Elabscience® Lactase Activity Colorimetric 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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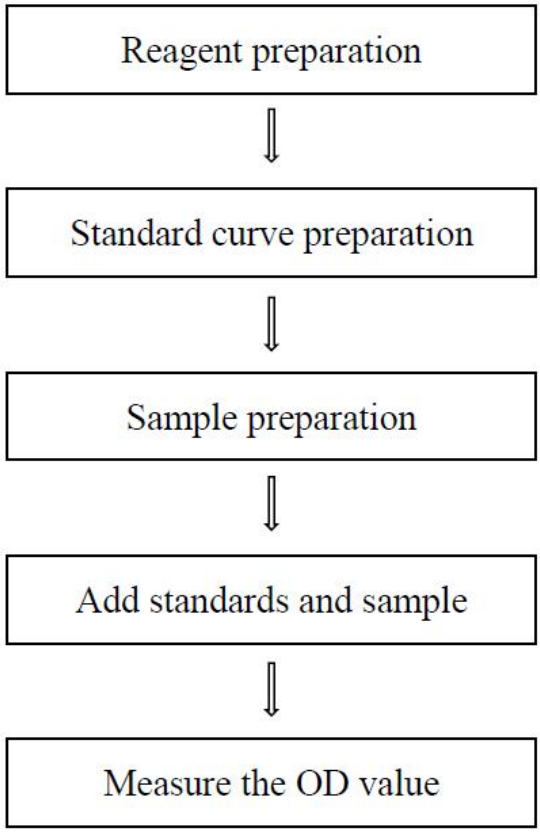
Assay summary



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. There is a characteristic absorption peak at 505 nm, and the magnitude of enzyme activity is directly proportional to the OD value.

Kit components & storage

Item	Component	Size (100 Assays)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder × 2 vials	2-8°C, 12 months
Reagent 3	Phenol Solution	28 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Enzyme Solution	28 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 5	50 mmol/L Glucose Standard	0.2 mL × 1 vial	2-8°C, 12 months

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:

Spectrophotometer (505 nm), Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25°C 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 working solution:
For each well, prepare 500 μL of chromogenic working solution (250 μL of phenol solution and 250 μL of enzyme solution). The chromogenic working solution should be prepared on spot and used up within 8 h protected from light.

④ The preparation of 5 mmol/L standard solution:
Before testing, please prepare sufficient 5 mmol/L standard solution. For example, prepare 1000 μL of 5 mmol/L standard solution (mix well 900 μL of double distilled water and 100 μL of 50 mmol/L glucose standard). Store at 2-8°C for 15 days protected from light.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**5.0**
5 mmol/L standard (μL)	0	40	60	80	100	120	140	200
Double distilled water (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Tissue sample:

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

② Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be detected within 5 h.

④ 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% Mouse small intestinal tissue homogenate	1-2
10% Mouse large intestinal tissue homogenate	1-2
10% Mouse kidney tissue homogenate	1
10% Mouse spleen 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.

Operating steps

① Standard tube: add 50 μL of standard solution with different concentrations to the corresponding 1.5 mL EP tubes.

Sample tube: add 50 μL of sample to the corresponding 1.5 mL EP tubes.

Control tube: add 50 μL of sample to the corresponding 1.5 mL EP tubes.

② Add 200 μL of substrate working solution to standard and sample tubes. Add 200 μL of buffer solution to control tubes.

③ Centrifuge at 500×g for 3 min.

④ Incubate at 37°C for 20 min protected from light.

⑤ Add 500 μL of chromogenic working solution to each tube.

⑥ Incubate at 37°C for 20 min protected from light.

⑦ Add 250 μL of buffer solution to each tube.

⑧ Set to zero with double distilled water and measure the OD values of each tube at 505 nm with 0.5 cm optical path cuvette.

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 enzyme in 1 g tissue protein per 1 min that hydrolyze 1 μmol of lactose at 37 °C is defined as 1 unit.

$$\text{lactase activity (U/gprot)} = (\Delta A - b) \div a \div T \div C_{pr} \times f \times 1000$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

T: Reaction time, 20 min

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

1000: 1 mmol/L=1000 μmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse kidney 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/L)	15.0	35.0	55.0
%CV	3.6	4.8	4.2

Inter-assay Precision

Three mouse kidney 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/L)	15.0	35.0	55.0
%CV	5.0	7	6.5

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 100.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	15.0	35.0	55.0
Observed Conc. (U/L)	14.9	35.7	55.0
Recovery rate (%)	99	102	100

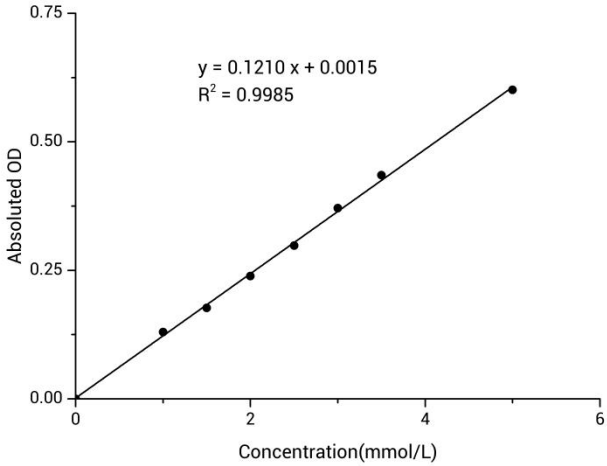
Sensitivity

The analytical sensitivity of the assay is 0.10 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	1.0	1.5	2.0	2.5	3.0	3.5	5.0
OD value	0.045	0.143	0.177	0.236	0.290	0.363	0.436	0.605
	0.045	0.116	0.176	0.241	0.306	0.378	0.433	0.597
Average OD	0.045	0.130	0.177	0.239	0.298	0.371	0.435	0.601
Absoluted OD	0.000	0.130	0.177	0.239	0.298	0.371	0.435	0.601



Appendix Π Example Analysis

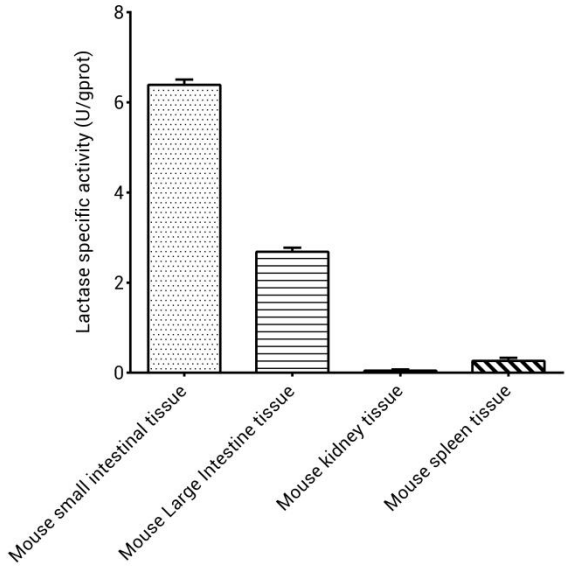
Example analysis：

Take 50 μL of 10% mouse small intestinal tissue homogenate and carry the assay according to the operation steps. The results are as follows: standard curve: y = 0.1210 x + 0.0015, the OD value of the sample is 0.278, the OD value of the control is 0.117, ΔA = 0.278 - 0.117 = 0.161. the concentration of protein is 5.20 gprot/L, and the calculation result is:

Lactase activity (U/gprot) = (0.161- 0.0015) ÷ 0.121 ÷ 5.20 ÷ 40 × 1000

= 6.34 U/gprot

Detect 10% mouse small intestinal tissue homogenate (the concentration of protein is 5.20 gprot/L, dilute for 2 times), 10% mouse large intestinal tissue homogenate (the concentration of protein is 4.58 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 11.78 gprot/L) and 10% mouse spleen tissue homogenate (the concentration of protein is 9.05 gprot/L), 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.