Technical Manual

Lactase Activity Assay Kit

- **Catalogue Code: MAES0106**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

12.5-2000 U/mL

Sensitivity:

3.94 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

Lactase is a glycoprotein with two active sites, which can catalyze the hydrolysis of various β-glycosidic bonds. It can catalyze the decomposition of lactose into glucose and galactose. Lactase plays a vital role in the nutrition of newborns in humans and other mammals, because this enzyme is the only small intestinal brush border hydrolase responsible for digesting lactose. Low levels of lactase and inability to digest lactose may cause lactose intolerance.

3. Intended Use

This kit can measure lactase activity in animal tissue samples.

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Substrate	Lyophilized × 1 vial	2-8°C, 6 months
Buffer Solution	10 mL x 1 vial	2-8°C, 6 months
Stop Solution	6 mL x 1 vial	2-8°C, 6 months
Phenol Solution	12 mL x 1 vial	2-8°C, 6 months, avoid direct sunlight
Enzyme Solution	12 mL x 1 vial	2-8°C, 6 months, avoid direct sunlight
Glucose Standard Solution (50 mmol/L)	1.5 mL×1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (495-510 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate working solution:** Dissolve a vial of substrate with 8 mL of buffer solution and mix fully. The prepared solution can be stored at 2-8°C for a month.

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

8. Sample Preparation

Tissue:
Weigh the tissue accurately and add normal saline at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement.

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 (12.5-2000 U/mL).

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);

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 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.

The measurement of samples

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.

Operation Table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	25		
Sample (μL)		25	
Substrate working solution (μL)	50	50	50
Mix fully and react at 37°C for 20 min.			
Stop solution (μL)	25	25	25
Sample (μL)			25
Mix fully and centrifuge at 1780 g for 10 min, take supernatant to corresponding wells in microplate.			
Supernatant (μL)	8	8	8
Chromogenic agent (μL)	200	200	200
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.			

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 of lactose hydrolyzed by 1 mg of tissue protein per minute at 37°C is defined as 1 unit.

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

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

f: Dilution factor of sample before tested

ΔA: $OD_{Sample} - OD_{Control}$.

20*: Reaction time, 20 min

1**000****: 1 μmol=1000 nmol.

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

12. Performance Characteristics

Detection Range	12.5-2000 U/mL
Sensitivity	3.94 U/mL
Average recovery rate (%)	102
Average inter-assay CV (%)	8.5
Average intra-assay CV (%)	4.5

Analysis

For rat lleal tissue, take 25 μL of 10% rat lleal tissue homogenate, carry the assay according to the operation table. The results are as follow.

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:

$$\text{Lactase activity (U/mgprot)} = \frac{(0.065 - 0.053 + 0.0078)}{0.0342} \div 20 \times 1000 \div 5.19$$

$$= 5.58 \text{ U/mgprot}$$

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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.

Notes:

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Notes:

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