TECHNICAL MANUAL

Lactase Activity Assay Kit

- **SKU CODE:** MAES0106
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples to tubes
- Incubate with substrate working solution
- Stop reaction and add samples to microplate
- Add chromogenic agent and measure OD at 505 nm

2. Intended use

This kit can measure lactase activity in animal tissue samples.

3. Detection principle

Lactase decomposes lactose to produce glucose. Under the action of enzymes, 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.

4. 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

Item	Component	Size (96 T)	Storage
	Plate Sealer	2 pieces	

5. 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)

6. 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. Store at 2-8°C for 1 month protected from light.
3. **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 immediately before use.
4. **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.

7. Sample preparation

1. **Tissue sample preparation:** 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 10,000×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 (MAES0177).
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 10% Rat ileal 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

2. If the lactase activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (MAES0177).

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

9. 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 thoroughly 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 thoroughly and centrifuge at 1,780×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 thoroughly for 10 s with microplate reader, incubate at 37°C for 15 min and measure the OD value of each well at 505 nm.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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°C is defined as 1 unit.

Lactase activity (U/mgprot) = (ΔA - b) ÷ a ÷ 20* ×1000** × f ÷ 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.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	25.00	103.00	254.00
%CV	8.2	8.6	8.7

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration tested 6 times in parallel to get the average recovery rate of 102%.

Recovery

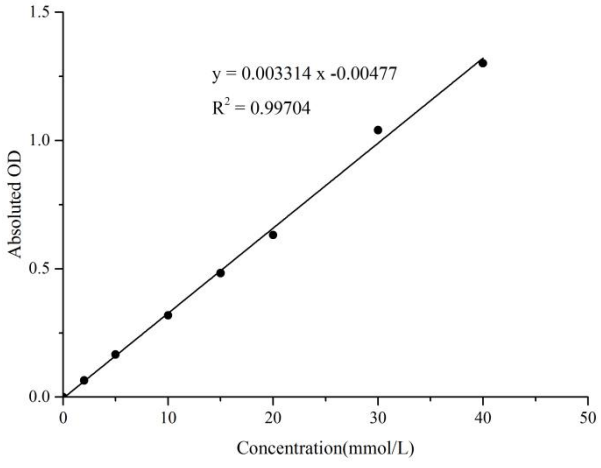
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	4.5	12	26
Observed Conc. (mmol/L)	4.5	11.9	27.6
Recovery rate (%)	101	99	106

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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mmol/L)	OD value		Average OD	Absolute OD
0	0.049	0.048	0.048	0.000
2	0.115	0.112	0.113	0.065
5	0.218	0.210	0.214	0.166
10	0.368	0.366	0.367	0.319
15	0.531	0.532	0.531	0.483
20	0.687	0.674	0.680	0.632
30	1.140	1.037	1.089	1.040
40	1.323	1.375	1.349	1.301

12. Appendix II Example Analysis

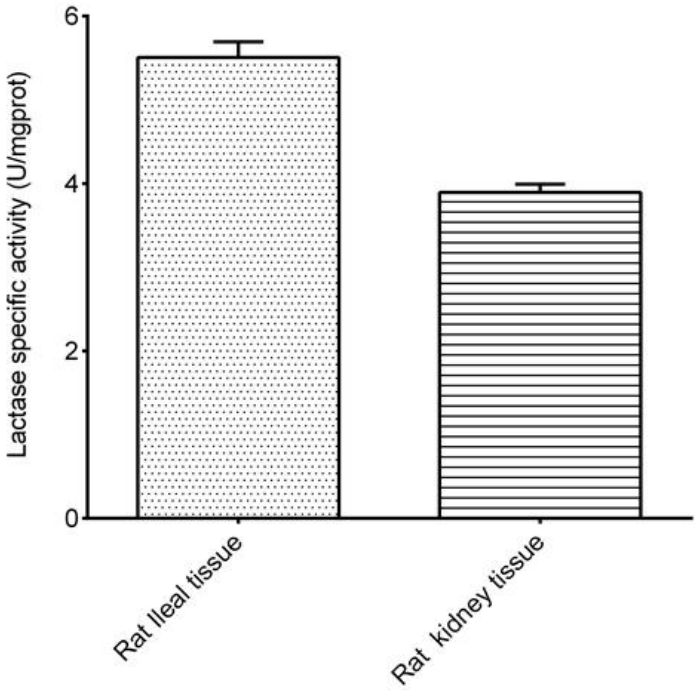
Example analysis:

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

Standard curve: y = 0.0342x - 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 ileal tissue homogenate (the concentration of protein is 5.19 mgprot/mL) and 10% rat kidney tissue homogenate (the concentration of protein is 9.50 mgprot/mL) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.